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Seawater use in agriculture:

a possible answer to reduce agricultural products' water footprint

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Cover photograph: particular of the common ice plant (Mesembryanthemum crystallinum L). photographed by Carlo Schiuma

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Chapter 1. General introduction:

Freshwater supply and food security

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Freshwater supply and food security

1.1 The virtual water concept

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1.1 The virtual water concept

The concept of virtual water - introduced by Professor Allan from King's College London in 1993 - defines the water needed to produce food, goods and services that we consume on a daily basis (Allan, 1993). In 2003, Arjen Hoekstra - the director of the Water Footprint Network - developed the concept of water footprint, connected to the virtual water one. It is an indicator of water consumption, applicable to individuals, communities or companies, which measures precisely the volume of water needed to produce food, goods and services consumed by individuals, communities or companies (Hoekstra et al., 2011). Providing some example, about 1830 liters of water are needed to produce a kilogram of wheat, about 2500 liters for a kilogram of rice (Mekonnen & Hoekstra, 2011) and approximately 15400 for a kilogram of beef (Mekonnen & Hoekstra, 2012). By calculating this amount of water, it is also possible to quantify the water that is exchanged between different countries, even if invisibly, with imports and exports of such goods.

Interestingly, the earth is covered for three quarters of its surface by water. Nevertheless, freshwater represents the smallest part, and an even smaller amount is available, in terms of accessibility and costs. Since water plays a central role both in nature and for all human activities, a possible decrease of its availability due to climate change and to the rising population is an urgent thematic to deal with. On top of that, the greater amount of water is demanded by food production. In fact, the agriculture sector is the most demanding one in terms of water resources (WWAP, 2012), reducing even more the gap between water availability and food security.

To better understand the different water components involved in food production, we here introduce the definitions of "blue water", "green water" and "gray water".

1. Blue water represents the surface water (i.e. rivers and lakes) or underground water. It is easily accessible and portable, it can be taken out, restrained, measured, distributed and stored (Antonelli and Greco, 2013). According to FAO estimates, 70% of this water is destined to worldwide irrigation (FAO, 2013). Blue water can be divided into two sub-categories: the water coming from renewable and from non-renewable sources. The first group includes the water surface and underground aquifers that are recharged by rainfall or snowmelt; the second group is represented by the water extracted from fossil aquifers, characterized by a minimum recharge percentage (Antonelli and Greco, 2013).

2. Green water includes rainwater or snow that do not turn blue, as it evaporates or is transpired by crops. It is not withdrawable or transportable as intrinsic to the rain-soil-plant system (Antonelli and Greco, 2013). Although not visible, the green component is the larger majority of the water used in agriculture (Fader et al., 2011). Interestingly, its use has a much softer impact on the environment compared to the use of blue water (Aldaya et al., 2010).

3. Gray water is the necessary water to dilute fertilizers or pesticides during the crop cycle, up to concentrations considered no longer harmful to the environment (Hoekstra et al., 2011); this component is therefore strictly dependent on the quantity and quality of input supplied during the production process.

The virtual water content of a food product, generally expressed in liters or cubic meters, is then given by the sum of its components: the green (water lost trough evapotranspiration during the entire production cycle), the blue (water supplied through irrigation) and the gray (the water used to dilute fertilizers and pesticides). However, we must point out that the "water sustainability" of agricultural food production is not to be attributed solely to the amount of virtual water contained in the product. In fact, it is the type of water used in the production phase to play the most important role. Accordingly, products obtained in rainfed agriculture are characterized by higher green water footprint compared to their blue water footprints. Also, such production is associated with a much softer environmental impact compared to goods produced from irrigated agriculture (Aldaya et al., 2010). In particular, because the green component is necessarily used in the agricultural sector (or preservation of the environment) and cannot, by its nature, have alternative uses. On the contrary, the blue component, being accessible and portable, could also be applied in different productive sectors (Antonelli and Greco, 2013). In fact, providing an example, although about 1000 liters of water are needed to produce 1 liter of milk (Mekonnen and Hoekstra, 2012), cattle reared on rainfed pasture produce milk with a much smaller water footprint compared to cattle feeding on irrigated forage.

We can conclude stating that all the agronomic practices aiming at water management are particularly essential today. Several practices are part of this group, from hydraulic facilities and irrigation systems to the selection of species with greater water use efficiency; from the erosion processes prevention to the improvement of water permeability and soil structure; from water conservation practices to the ability of recycling waste water or desalinate sea water. Moreover, climate change consequences on water resources are already turning into drought conditions in certain areas of the world (i.e. in the Mediterranean), whereas in other countries (for example in South-East Asia) are leading to a considerable rainfall increase. The ability of making the soil more capable of capturing water, may it be a little amount or an over quantity, to be thus usable by the crop, has always been an important concern of farmers from all over the world. Interestingly, those aspects maintain their importance nowadays, as well as in the future.

The analysis of virtual water and water footprint concepts, representing an unseen but decisive amount of water which is hidden inside alimentary goods, may be a valuable tool for the analysis of the sustainability of agricultural food production systems. Furthermore, such concepts might also help in understanding the climate change effects on water resources. In the end, these concepts identify the necessary elements to assess the virtual water trade - "hidden" in the export and import of agricultural and food goods - globally. In particular, the virtual water trade will be more beneficial when exports go towards "water poorer" countries or when the goods exported are characterized by high green water footprint. On the contrary, such trade might be recognized as harmful, thus potentially correctable, if production overexploits local water resources, with direct consequences against both the environment and the populations of the exporting country.

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Chapter 1. General introduction:

Freshwater supply and food security

1.2 Seawater in soilless culture: a review

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1.2 Seawater in soilless culture: a review

Abstract

Water is the engine of life. All organisms, human included, are largely made of it. Of all the water on the planet, seawater represents about 97%. Almost 2% of the remaining fresh water is locked in ice. Accordingly, available freshwater counts on less than 1% and of such share an average of 70% is absorbed by the agricultural sector. Since the rhythm of freshwater withdrawal is faster than its regeneration, measures have to be taken in order to preserve such an important resource. The use of seawater in soilless culture is an interesting option to limit freshwater withdrawal, with no additional negative imput into soils. The present review aims at describing the state of the art of seawater as an irrigation complementary source in soilless culture, providing a critical overview on the available information on the subject.

1. Introduction

Water is linked with life, all organisms requiring it and the majority, human included, being largely made of it (Brown, 2011). Interestingly, water naturally recycles through the water cycle, a continuous cycle where water evaporates and falls down again as precipitation, then evaporating again. Belonging to the renewable, though finite, resources, the Earth has lot of water on its surface and in the ground just below: about 1.4 billion cubic kilometers in total (FAO, 1995). Of this amount, seawater represents about 97% and almost 2% of remaining freshwater is locked in ice. Accordingly, available freshwater counts on less than 1% (FAO, 1995), and between the many uses to which it is destined, the agricultural sector absorbs an average of 70% (FAO, 2011), reaching 90% in the Middle-East and North Africa (MENA) regions (FAO, 1988; SDSN, 2013). Since the rhythm of freshwater withdrawal is faster than its regeneration, measures have to be taken in order to preserve such an important resource. The controlled use of seawater in soilless culture could limit, with any negative imput into soils, the freshwater withdrawal destined to food production.

Seawater in fact is the most abundant source of water of the planet. Therefore, it is emerging as a real possibility in the agricultural sector, either desalinized or blended with other water sources, i.e. brackish waters (Yermiyahu et al., 2007). Interestingly, seawater specific composition represents a very well balanced ionic environment for plants (Boyko, 1966): in fact, despite its very high chloride content (about 75% NaCl and 10% of $MgCl_2$), seawater is rich in all nutritive elements needed by plants, including the necessary trace elements and micro-organisms, living or dead (Eyster 1968, Boyko & Boyko 1966). Moreover, in the majority of species, plant salt tolerance when treated with seawater seems to be multiplied compared to NaCl solutions treatments at comparable electrical conductivity (EC) (Boyko and Boyko, 1966), NaCl solutions representing instead the most used treatments in trials investigating crops salt resistance in worldwide scientific literature. Yet, seawater use in agriculture is studied since the early sixties of the last century (Boyko, 1966). According to Breckle (2009), a large scale sustainable agriculture relying on pure seawater is not feasible for several reasons, among which the sodium concentration of full strength seawater resulting in toxic effects for the majority of species and determining the deterioration of soil structure, especially without an efficient leaching system. Nevertheless, in other cases, small-scale seawater irrigation may be suitable and economically viable, as for example on anyhow saline coastal areas (Breckle, 2009) or in soilless culture. In fact, quoting the words of prominent experts: the heart of new agricultural paradigms for a hotter and more populous world must be powered and irrigated as much as possible by sunlight and seawater (Fedoroff et al., 2010). The present review aims at describing the state of the art of seawater as an irrigation complementary source in soilless culture, providing a critical overview on the available information on the subject.

2. Soilless culture and water saving

Soilless culture is defined as "any method of growing plants without the use of soil as a rooting medium, in which the inorganic nutrients absorbed by the roots are supplied via the irrigation water" (Resh, 2012). It is considered as a modern practice, but growing plants with similar techniques has been often tried throughout the ages, probably first by Egyptians several hundred years B.C. (Hussain et al., 2014; Raviv and Lieth, 2008). In fact, soilless systems offer an important alternative to soil cultivation in case of soil and/or water issues, as for example, among the most important, water shortage and salinization (Olympios, 1999). The three main soilless systems are liquid hydroponics, solid media culture and aeroponics. Hydroponics is further categorized in open or closed systems, depending on the collection and reuse (i.e. in closed systems) of the nutrient solution until its depletion. Solid media culture systems can in the same way be open or closed and several substrates are used for plants anchorage (i.e. perlite, vermiculite, coconut coir), as long as characterized by water and air holding capacity and by an easy drainage. Aeroponics, in the end, enables the maximum utilization of space by growing plants with roots suspended in air sprayed every 2-3 minutes, plants getting nutrients and water from the solution film that adheres on roots (Hussain et al., 2014). This diversity of techniques makes soilless culture adaptable to very dissimilar situations, with the common potential application in providing food in areas characterized by soil and water availability issues (Sheikh, 2006).

Indeed, a number of crops can be grown on commercial level in soilless culture, principally fruits and vegetables. Examples are *Fragaria ananassa* (Strawberry), *Lactuca sativa* (Lettuce), *Lycopersicon esculentum* (Tomato), *Phaseolus vulgaris* (Green bean), *Beta vulgaris* (Beet), *Cucumis sativus* (Cucumbers), *Cucumis melo* (Melons), *Allium cepa* (Onion) and many others (Hussain et al., 2014). Thus, soilless culture can play an important role in providing essential vitamins, minerals, and dietary fibre (FAO, 2003). Even more, it could help in fulfilling the recommended minimum intake of fruits and vegetables per person, food categories which are nowadays not consumed enough in several world regions (WHO and FAO, 2003), with reasons to be found in lack of tradition in their cultivation and use (Orsini et al., 2013), or because appropriate environment requirements (i.e. soil and water) are missing. Thus, soilless systems can promote diversification of crops and species, hence of consumption behaviors.

Interestingly, considerable amounts of water are saved in soilless culture compared to soil cultivation. In fact, no percolation losses occur when growing plants with roots directly in contact with the growing media. Moreover, water for soil leaching is in this case unneeded. Such amount of water depends on several parameters: site-specific estimates of total precipitation, irrigation, evapotraspiration, soil bulk density and porosity, solute characteristics and distribution (Baes and Sharp, 1983), all characteristics that do not overall influence the amount of required water in soilless agriculture. Because of these reasons, the saving of about 10-30% of water compared to soil cultivation is achievable in soilless culture (Sheikh, 2006), especially with the closed-recirculating systems characterized by no drainage nor evaporation from the surface (Olympios, 1999). Accordingly, the water use efficiency of soilless plant production is higher than that of soil-grown plants (Raviv and Lieth, 2008) and an overall more accurate control over the supply of water is practiced (Olympios, 1999). Besides, it is interesting to note that salinity is less dangerous on soilless grown plants compared to soil cultivation, because roots ability of excluding toxic ions (i.e. Na^+) and of withstanding high osmotic pressure is enhanced by the full oxygen supply to roots, which are more oxygenated then in soil conditions (Raviv and Lieth, 2008). Soilless culture can thus contribute to limit the freshwater withdrawal of the agricultural sector, assuring food production with a considerable reduction of freshwater use, and possibly making productive alternative sources of water, i.e. seawater.

3. Seawater as a complementary irrigation source

Literature offers plenty of experiments testing crops responses to salinity stress. Even if salt stress is administered through NaCl solutions in the majority of trials, the number of experiments using diluted or full strength seawater is growing. Both considering the experiments testing plants salt response by using NaCl solutions or seawater, the general and firstly observed effect of salinity is the reduction of plants growth rate (Roy et al., 2014), this decrease changing between species and varieties (Shannon and Grieve, 1998). Such growth reduction easily translates to yield reduction. Reasons are to be found in many factors, and between the most important: water and osmotic stress, ion toxicity, nutritional disorders, oxidative stress, metabolic processes alterations, membrane disorganization and genotoxicity (Munns, 2002). In fact, plants major processes (i.e. photosynthesis, protein synthesis and energy and lipid metabolism) are affected (Parida and Das, 2005) and the salt-induced osmotic potential diminution limits the water availability for the plant, inducing water stress consequences (West et al., 1986; Yeo et al., 1985). Moreover, ion toxicities and/or nutritional deficiencies can occur if a specific ion prevails or because of competition effects between different ions (Bernstein et al., 1973). Importantly, as mentioned before, seawater salt tolerance, for the majority of species, is considerably higher than NaCl salt tolerance (Boyko and Boyko, 1966).

Accordingly, this review is focused on the studies testing crops grown with seawater (full strength or at different concentrations/dilutions). Overall, researchers working on this topic concluded that seawater in the growing media, up to certain concentrations, does not negatively affect the productivity. Maximum thresholds are to be found according to the species or to the variety cultivated (Shannon and Grieve, 1998). Moreover, salt tolerance is generally enhanced when plants are not suddenly shocked, but allowed to gradually acclimate to salt stress (Noaman et al., 2002; Parida and Das, 2005). In fact, acclimation allows plants to better cope with salinity with several physiological, biochemical and molecular strategies. Among the most important: the accumulation, exclusion or compartment of ions; the synthesis of osmotic solutes to keep the ionic balance in the vacuoles; the enhanced production of antioxidant compounds and plants hormones (i.e. ABA and cytokinins); the expression of early salt induced (ESI) genes in the roots (Noaman et al., 2002; Parida and Das, 2005). Increasing salt tolerance by acclimating plants could extend seawater use in the growing media both reaching higher maximum thresholds of seawater tolerance, and including also less tolerant species to be potentially grown with seawater. Besides, as mentioned before, several quality improvements in plants grown with diluted seawater, originated by plants adaptation to salinity, were generally assessed: certain plant secondary metabolites are in fact accumulated in response to stress condition, as an adaptation to the environment (Ramakrishna and Ravishankar, 2011). To provide some examples, anthocyanins, polyphenols and proline content are known to increase accordingly with increasing salinity (Parida and Das, 2005). Interestingly, the increase of the endogenous concentration of such compounds, with high nutritive properties, can be precious for the nutritive characteristics of food (Sgherri et al. 2008; Di Baccio, Navari-Izzo, and Izzo 2004). Salinity, in some cases, may then result to be favorable for yield and its quality (Shannon and Grieve, 1998).

3.1 *Tomato crop*

Numerous experiments were performed in the last decades on tomato crop (*Solanum lycopersicum* L.), classified as a moderate salinity-tolerant species (Maas and Hoffman, 1977). Tomato is in fact cultivated all over the world and growers of many areas are facing water shortage or deterioration (Sgherri et al. 2007), i.e. water salinization. It is from the 70's that tomato crop is studied in relation to its salt tolerance. Rush & Epstein (1976), for example, investigated salt-sensitive and salt-tolerant (i.e. ecotypes from the Galapagos Islands) genotypes of tomato assessing a far stronger salt resistance in the Galapagos ecotypes which were surviving in a full strength seawater nutrient solution (EC roughly corresponding to 50 dS m^{-1}), while the salt-sensitive cultivar could not in most cases withstand levels higher than 50% seawater (Rush and Epstein, 1976). The tentative of transferring genetic information from the salt-resistant ecotypes to cultivated tomato by breeding did not lead to cultivars suitable for commercial growers. Thus, subsequent research focused on the determination of physiological traits differences among accessions characterized by different salinity tolerance thresholds that might be useful in future breeding programs. Among the main results, succulence and good selectivity for potassium over sodium proved to be important characteristics associated to salt-tolerance in tomato (Cuartero et al., 1992). To date, breeding salt-tolerant tomato cultivars faced many obstacles, mainly because traits related with salt tolerance are not combined in a single donor genotype (Cuartero and Fernandez-Munos, 1999). Therefore, more information about the traits related to the desired features is still needed.

Along with the unsuccessful breeding effort, several trials aimed at determining the seawater tolerance threshold of cultivated tomato assessing, in the meanwhile, the characteristics of fruits. Overall, even if seawater irrigation generally reduced the crop yield, many studies concluded that irrigating tomato with seawater concentrations adapted to the tested variety, in particular between 10 to 20% seawater (roughly EC of 8 dS m^{-1} to 14 dS m^{-1}), increased the nutritional value of the product. For example, despite the reduction of fruit yield (kg/plant), fruits dry matter and total soluble solids were reported to increase by several authors with seawater concentrations of 10% and 12% compared to control. Importantly, fruits were all marketable, and the increased fruits dry matter and total soluble solids made them particularly desirable for the canned tomato industry, being these traits able to improve the quality of the processed product (Sgherri et al. 2008; Sgherri et al. 2007; De Pascale et al. 2001). In addition to that, the concentration of reducing sugars (RS) and titratable acidity (TA) also increased in the berries exposed to seawater irrigation, thus resulting tastier then control ones (Sgherri et al. 2008). A similar significant increase was observed by Ullah et al. (1994) in a pot experiment in seawater irrigated tomato fruits compared to control fruits. In particular, glucose, fructose, citric and ascorbic acid increased proportionally to salinity, with glucose concentrations up to 139% and fructose up to 101% above the control treatment. Accordingly, salt stress turned out to enhance both the sweetness and the quality of fruits (Ullah et al., 1994). Again, Sgherri and collaborators (2008; 2007) reported the increase of many other improving quality compounds in 10%-12% seawater treated tomatoes: NADPH, $NADPH/NADP^+$, ascorbic acid (Vitamin C), α-tocopherol (the main representative of Vitamin E in tomato, according to Seybold et al. 2004), total ascorbate, dihydrolipoic: all resulted to be increased compared to control tomatoes (Sgherri et al. 2008; Sgherri et al. 2007). Indeed such compounds linked to the nutritional value of the product are related to oxidative processes depending by natural physiological processes (i.e. ripening), but they can also be enhanced by environmental changes, as in this case by salinity conditions (Sgherri et al. 2008). In fact, the enhanced synthesis of antioxidants is considered as one of plants adaptive mechanisms to biotic and abiotic stresses (Sgherri et al. 2008). Also, priming seed for 36 hours with 1M NaCl proved to extend salt tolerance of the grown plants (Cuartero and Fernandez-Munos, 1999). Overall, major differences in the response to salinity are observable among different varieties: interestingly, certain of those show promising response to seawater irrigation by producing more yield compared to non saline conditions (Al-Busaidi et al., 2009).

3.2 *Leaf vegetables*

Leaf vegetables (i.e. lettuce, chard, chicory) have being deeply tested under seawater irrigation in soilless systems. Lettuce (*Lactuca sativa* L.) growth, for instance, was tested under different seawater concentrations, up to 20% (roughly EC 14 dS m^{-1}) (Atzori et al., 2016; Lee et al., 2011; Sakamoto et al., 2014; Turhan et al., 2014). Different results obtained in trials testing different cultivars indicate certain traits to be probably cultivar-dependent. Focusing firstly on growth, *Lactuca sativa* cv. Mother-red growth was not negatively affected by seawater up to EC 10.6 dS m^{-1} (Sakamoto et al., 2014), whereas *Lactuca sativa* L. cv. Canasta resulted in a reduced growth starting from EC of 6.12 dS m^{-1} (Atzori et al., 2016) and cv. Funly from EC of 3.7 dS m^{-1} (Turhan et al., 2014); in those experiments, the concentration of nutritionally important mineral elements, such as K^+, Ca^{2+}, Cu^{2+} and Mg^{2+}, increased accordingly with increasing salinity. Sakamoto and collaborators (2014) also observed a significant seawater-related increase of the concentration of anthocyanins, carotenoids and sugar content in leaves, whereas results on cv. Canasta did not report significant changes in polyphenols and carotenoids, and soluble sugars decreased with increasing salinity compared to control (Atzori et al., 2016). In Turhan and collaborators pot experiment (2014), lettuce cv. Funly was irrigated with 0%, 2.5%, 5%, 10%, 15%, and 20% seawater: total soluble solids, total sugar, and protein content significantly increased at low salinity levels (2.5% and 5%) while decreased with higher seawater compared to control. Despite the important differences among cultivars in their degree of salt tolerance, as several compounds known for their healthy properties and organoleptic qualities (i.e. antioxidants, soluble solids, sugars, mineral elements) are linked to the plant defense system, the possibility of enhancing their concentration by growing crops with diluted seawater deserves attention (Sgherri et al. 2008).

Among leaf vegetables, chard (*Beta vulgaris* L.) and chicory (*Cichorium intybus* L.) crops were successfully grown with 15% seawater, thus up to EC values of 9.2 dS m^{-1} (Atzori et al., 2016; Zhang et al., 2008, 2009). These crops proved to have a considerable higher tolerance to salinity compared to lettuce. Such tolerance is probably connected with their salt resistant ancestors, having possibly inherited from them salt resistance traits (Shannon & Grieve 1998). For example, the wild sea beet (*Beta vulgaris* subsp. maritima) is believed to be the ancestor of both leaf and root beets. Similarly, *Cichorium intybus* is native of the Mediterranean region, where it is easily found as weed in saline areas (Boyd and Rogers, 2004; Shannon and Grieve, 1998). Interestingly, along with the unreduced growth performances, seawater in the growing media lead to an increase of a number of mineral elements in such crops: Ca^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, Zn^{2+} in chard leaves and K^{+} and Cu^{2+} in chicory leaves (Atzori et al., 2016), thus representing an interesting case of biofortification obtainable in saline conditions. Likewise, Na^{+} increased accordingly with seawater: this parameter needs attention as it can result in toxicity effects if consumed beyond certain amounts.

3.3 *Non-food crops*

Along with trials focusing directly on edible fruits or leaves, other species have been tested to understand the biochemical and physiological mechanisms beyond seawater adaptation of the whole plant, with the aim of improving and/or selecting crops with salt tolerance traits (Di Baccio et al., 2004). For example, an experiment on sunflower (*Helianthus annuus* L.) grown with 10% and 20% of seawater investigated the oxidative responses of the plant: different responses to the two seawater concentrations were assessed. In particular, growth reduction, due to oxidative stress, was observed at 20% whereas 10% seawater grown plants did not differ from control. In both cases, interestingly, plants were able to regenerate antioxidant molecules, with considerable differences between roots and shoots (Di Baccio et al., 2004). Also, Jin and collaborators (2007) assessed the possibility of growing *Aloe vera* L. up to 60% seawater: its main osmotic adjustment mechanism was found to be the accumulation of inorgatic cations in roots. Besides, five ecotypes of the Jerusalem artichoke (*Helianthus tuberosus* L.) were grown in hydroponics at 15% and 30% seawater and several molecules resulted to be stimulated in plants exposed to seawater: antioxidant enzimes, organic acids, proline, soluble sugars and inorganic solutes, even if with important ecotypic variabilities (Xiao-hua et al., 2009).

4. Potential and constraints

Definitive conclusions on crops salt tolerance are hard to be found because of many reasons. Between the most important: the remarkable differences that exist among species and varieties; environmental factors, such as temperature and humidity, that may affect plants ability to cope with salt stress too; plants can adapt to environmental conditions, making what was found for a population not necessarily true for the next one. Along with this, literature is far more abundant on NaCl experiments then on seawater ones, two types of salinity stresses that may not always be comparable, as in the majority of species salt tolerance when treated with seawater seems to be multiplied compared to salt tolerance to NaCl solution at comparable EC values (Boyko and Boyko, 1966). It seems then complex to obtain any definitive a priori screening on crops salt tolerance. In fact, even if plant resistance to salinity is under study since more than a century in several scientific sectors, this topic is nowadays still unanswered and prevailing. Discoveries in this field might potentially offer new productive methods focusing on the preservation of freshwater and, in the meantime, on the sustainable exploitation of an abundant resource as seawater.

In fact, the potential of seawater irrigated soilless culture is mainly represented by the most accurate control over the supply of water. Soilless culture per se assures an important increase of the water productivity in the cropping system (Olympios, 1999). Adding seawater as a controlled complementary source of irrigation may have a twofold aim: it allows the saving of a significant amount of freshwater while exploiting the seawater nutrient content. Also, in certain cases, the enhancement of several plant metabolites, precious for human consumption, could be obtained. On the other hand, major constraints are linked to the increases of sodium and chlorides (Ullah et al., 1994), which can have toxic effects if assumed in high concentrations. Individuating the crop-specific seawater concentration that balances the just described potentials and constraints has thus crucial importance.

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Chapter 1. General introduction:

Freshwater supply and food security

1.3 Halophytes as potential saline crops in coastal environments

1.3 Halophytes as potential saline crops in coastal environments

Agriculture is at the center of global efforts of adaptation to climate change because of two main reasons: it is essential for our food supply and it depends directly on natural resources, which are inextricably linked to climate (FAO, 2016). In particular, coastal areas are among the most vulnerable zones to climate change consequences: serious problems are in fact forecasted because of the sea level increase and tidal surges which can easily translate into inundation and consequent submergence of coastal wetlands (Flowers and Muscolo 2015). In fact, as a result of global warming, sea level rise is already an issue in coastal areas (Nicholls and Cazenave, 2010) and nearby arable lands, which productivity is showing reductions because of increasing salinization (Lee and Song, 2007). Moreover, the current demographic growth rate will lead to a world population of up to 10.9 billion people by the end of the century (UN, 2013). As a consequence, an increase of about 70% of food production is needed to feed the future world population (FAO, 2016, 2009). Agriculture itself, on the other hand, contributes to climate change through the greenhouse gas emissions that are linked to the increase of global warming (FAO, 2016). In fact, agriculture (including forestry and other land use) contributes to 21% of total greenhouse gas emissions (FAO, 2016). New sustainable agricultural systems are thus necessary to adapt to current or expected climate changes, minimizing their negative effects and possibly taking advantage of the opportunities created (FAO, 2016). Accordingly, saline agriculture could represent a climate change adaptation strategy.

Saline agriculture implies the cultivation of crops through irrigation with brackish and seawater (Rozema and Schat, 2013). This approach seems ecologically optimal in coastal environments, where the use of seawater would not negatively affect the soils structure and fertility, as such soils are naturally exposed to saline conditions. In fact, the use of saline waters is not suggested in soils where salinity is not already a permanent issue, because it would lead to important soil losses in structure and fertility. However, most cultivated crops do not fit with a saline agriculture context, being the majority of them salt sensitive. Above certain seawater salinity thresholds, varying widely among different species and varieties (Shannon and Grieve, 1998), major yield reductions are observed, due to water stress, ion toxicity, nutritional disorders, oxidative stress, metabolic processes alterations, membrane disorganization and genotoxicity among other factors (Munns, 2002). Salt in fact represents one of the major abiotic stresses affecting crops yield wide world (Redwan et al., 2016). On the other hand, coastal areas could support the cultivation of edible species native from salt marshes and inland saline sites, thus naturally adapted to high saline environments.

Interestingly, about 50000 edible plants grow on the Earth. Nevertheless, only a few hundred are cultivated as crops (FAO, 1995). It seems thus possible to introduce new crops for human nutrition, contributing to the diversification of food consumption. Besides, such an high number of edible species represents an important genetic resource, with major role in building resilient agricultural production systems: new crop varieties will in fact be needed to take account of the occurring changes and to provide adaptation for future changes (FAO, 2016, 2015). This may be a way for agriculture to rethink several of its schemes accordingly with the new and changing climatic conditions, in order to decrease its vulnerability to risk events and to reach the food security goal.

Halophytes are defined by Flowers & Colmer (2008) as salt tolerant plants capable of growth and reproduction at soil salinities greater than 200 mM NaCl: such value roughly corresponds to a salinity greater than the one of half strength seawater. Halophytes can grow and reproduce on saline soils that kill 99% of the other species (Flowers and Colmer, 2008) and they are estimated to consist in 5000 to 6000 species, or 2% of total world angiosperm species (Glenn et al., 1999). They are spread over numerous plant orders; dicotyledonous halophytes, more numerous then monocotyledonous species, are present in 16 different orders (Flowers et al., 2010). Among those, the most represented is Caryophyllales, with the largest number of halophyte species in the Chenopodiaceae family, with over the half of its 550 species. Also Poaceae, Fabaceae, and Asteraceae count remarkable numbers of halophytes (Glenn et al., 1999).

Adaptation of halophytes to saline environments may be characterized as salt tolerance or as salt avoidance (Koyro et al., 2011). A wide range of morphological, physiological, and biochemical adaptations exists in halophytes, varying widely in their degree of salt tolerance (Flowers and Colmer, 2008). Overall, halophytes use the controlled uptake of Na^+ into cell vacuoles to be able to uptake water despite a low external water potential (Glenn et al., 1999). Other characteristic mechanisms do exist in certain halophyte species, as for example the salt excreting glands developed on the leaves of the black mangroves (*Avicenia* spp.), which excrete the excess salts out of the leaf surface (Popp et al., 1993), or the large epidermal bladder cells of *Mesambryanthemum crystallinum* L., which sequester salt and function as peripheral salinity and water reservoirs providing protection from short term high salinity or water deficit stress (Agarie et al., 2007; Luttge et al., 1978). The first modern researchers suggesting high salinity agriculture were Hugo and Elisabeth Boyko (Boyko, 1966) and since then important experiments aimed at investigating such a possibility. Among the main important we recall the works of Ayala and O’Leary, 1995; Boestfleisch et al., 2014; Bruning et al., 2015; de Vos et al., 2013, 2010; Katschnig et al., 2013; Koyro, 2006; Panuccio et al., 2014; Rozema et al., 2015. Many reviews also depth the subject, and among the most relevant: Bruning and Rozema 2013; Flowers and Muscolo 2015; Glenn et al. 1999; Koyro et al. 2011; Rozema and Flowers 2008; Ventura et al. 2015. Interestingly, many of those salt tolerant plants are edible, thus their domestication and cultivation in a saline agriculture context could be regarded as an interesting approach to consider (Glenn et al., 1998; Rozema and Flowers, 2008; Rozema and Schat, 2013; Ventura et al., 2015), allowing the exploitation of the great availability of brackish and seawater and of its important content of macro and micronutrients that are essential for plants growth (Rozema and Flowers 2008).

Besides, plants growing in saline environments are often associated with enhanced endogenous concentrations of high-nutrient compounds. In fact, the numerous biochemical strategies adopted by plants to cope with salinity include the selective accumulation or exclusion of ions; the synthesis of osmotic solutes; the induction of antioxidant compounds (Parida and Das 2005), or secondary metabolites. Providing some examples, anthocyanins, polyphenols and proline content are known to increase accordingly with increasing salinity (Parida and Das, 2005). These compounds, referred also as plant stress metabolites, namely produced by plants to interact with the environment, in terms of adaptation and defense (Ramakrishna and Ravishankar, 2011), are known to have important roles in human nutrition. Overall, halophytes show enhanced stress-related metabolites as compared with glycophytes in their natural habitats and a change of conditions can further enhance such compounds concentration (Flowers and Muscolo 2015). For example, it was demonstrated that it is possible to increase the antioxidant capacity of several halophyte species, i.e. *Tripolium pannonicum*, *Plantago coronopus*, *Lepidium latifolium* and *Salicornia europaea*, by modifying the saline growing environment and the length of saline cultivation (Boestfleisch et al., 2014). Osmolites and secondary metabolites can be used as functional food, defined as having disease-preventing benefits (Ventura et al., 2015). Accordingly, the increase of the endogenous concentration of such compounds can be precious in food production because of their high nutritive characteristics (Di Baccio et al., 2004; Sgherri et al., 2008). Also, the modern attention to healthier diets may promote new markets for halophytes with high nutritive potential (Ventura et al., 2015), corroborating the saline agriculture opportunity.

A number of halophytes are already consumed in several countries as spontaneous plants. Also, because of their diversity, numerous species have been tested as vegetables, forage and oilseed crops (Koyro et al., 2011). Nevertheless, as reported by Glenn and collaborators (1999), the following requisites have to be met in a saline agriculture context cultivating halophytes:

a) the cultivated halophytes must have a high yield potential;

b) the irrigation requirements must not damage the soil;

c) halophyte products must be able to substitute (or integrate) conventional crop products.

a) Despite the numerous laboratory experiments conducted on halophytes, scientific documentation at field conditions is very scarce. Hence, there is a strong need for large-scale field experiments to evaluate the feasibility of halophyte crop production, together with the economic perspective for future growers (Ventura et al., 2015). For those evaluations, facilities like the Salt Farm Texel (Figure 1) in the Netherlands proved to be optimal.

Figure 1. Salt Farm Texel, The Netherlands

Salt Farm Texel is a farm provided with a research station of one hectare under field conditions where freshwater and seawater can be mixed together into seven different salt concentrations. In total 56 plots are irrigated, with each salinity level repeated eight times and randomly distributed. Several halophytes are grown and studied in such facilities on behalf of governments, water boards, universities and companies, among many others (Salt farm Texel).

b) As mentioned before, sandy coastal areas would not be damaged by the use of seawater as irrigation source. In fact, coastal areas are naturally affected by seawater infiltration and salinity is the main factor for poor crop yield (Kaur et al., 1998). Also, soils in coastal areas generally have a sandy texture, thus very porous, allowing the best percolation of salts in the interest of the cultivated halophyte species (Boyko and Boyko, 1966). The right choice of cultivation systems has major importance: on fertile soils salt contamination would occur through Ca^{2+}/Na^{2+} exchange and consequent clay dispersion, whereas sandy soils of coastal areas may be available for large-scale halophyte production without the risk of salt contamination (Ventura et al., 2015).

c) Certain halophytes are already consumed in several countries of the world, mainly as vegetables or as oil source. They can substitute conventional crops, but they do not compete with glycophytic food production (Ventura et al., 2015), as different natural resources are used in the production. Certain species, i.e. *Salicornia europaea*, also started to appear in popular Italian supermarkets, even if misclassified as "seaweeds". In The Netherlands, the products of Salt Farm Texel (i.e. *Salicornia europaea* and *Mesambryanthemum crystallinum*) are trademark-registered products and sold in supermarkets. Also, even if the majority of consumption of such species is still linked to the popular culture, they are being rediscovered also by chef of excellence, as for *Crithmum maritimum* L. used by the chef Heinz Beck in his recipe "The Water Garden" (Figure 2).

Figure 2. The Water Garden of Chef Heinz Beck

After all those considerations, we can say that the goals pursuable by saline agriculture are numerous. To begin with, an increased sustainable food production could be achieved exploiting resources which would not be used for conventional agriculture: (1) seawater or brackish waters as complementary irrigation waters and (2) salt-affected soils (Glenn et al., 1999). Furthermore, halophytes grown in saline conditions could be a source of compounds with a potential nutritive and economic value (Flowers and Muscolo, 2015). Of course, future perspectives include more research with halophytes. In particular, large-scale and long-term experiments evaluating the sustainability of halophytes crop production are needed, together with cultivation protocols that do not exist for halophyte crops (Ventura et al., 2015); physiological and molecular studies can contribute to the understanding of the physiological and biological mechanisms behind the halophytes' salt tolerance; ecological studies need to assess saline agriculture in coastal areas as an ecologically acceptable and sustainable option; investigations on the products quality are needed to approve their nutritional composition and functional value for human alimentation; the development of halophytic agriculture would also need to be undertaken together with studies of hydrological and soil management of saline agriculture systems (Rozema and Flowers, 2008).

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Chapter 1. General introduction: Freshwater supply and food security

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1.4 Aims and outlines of this thesis

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This thesis aims at investigating the possibility of using seawater in agriculture as a complementary or alternative water source. The purpose is to understand if its introduction can help in reducing the agricultural products' water footprint and thus the need in water of food production. Seawater represents 97% of the total amount of water of the Earth, whereas the available freshwater counts less than 1% (FAO, 1995). Of such a small share of fresh water, food production requires on average 70% (FAO, 2011), reaching up to 90% in the Middle-East and North Africa (MENA) regions (SDSN, 2013). Furthermore, a remarkable increase in food production is forecast because of the growing population; such necessity will tremendously increase the human pressure on the water resource (FAO, 2016, 2009). On the other hand, as just mentioned, seawater is the most abundant source of water of the planet. Interestingly, its specific composition represents a very well balanced ionic environment for plants (Boyko, 1966): in fact, with the exception of its very high chloride content (about 75% NaCl and 10% of $MgCl_2$), seawater is rich in all nutritive elements needed by plants, including the necessary trace elements and micro-organisms, living or dead (Eyster 1968, Boyko & Boyko 1966). If production is still competitive by replacing a part of fresh water with seawater, its use in agriculture could represent an answer to limit the use of freshwater and, in the meanwhile, to exploit seawater as a nutrient supply for plants growth.

In this thesis such possibility is investigated both on glycophytes and halophytes, focusing on edible species only. Because of the important differences in salt tolerance between the two groups, the experiments conducted used different shares of seawater to grow plants, corresponding to maximum electrical conductivity values of 9 dS m^{-1} for glycophytes (Chapter 2) and of 35 dS m^{-1} in the experiment testing the halophyte species (Chapter 4). Also, glycophytes were tested in hydroponic conditions (Chapter 2 and Chapter 3): in this way, any excess in salts was prevented to enter in the soil ecosystem, not to generate loss of structure and fertility. The aim of the experiments on glycophytes was to identify the maximum threshold of seawater that different species can tolerate without any loss in productivity. On the opposite, the experiment on the edible halophyte reported in Chapter 4 was a field experiment. The tested species was grown in the coastal area of the Texel Island in The Netherlands, on soil which mainly consists of sand. Such choice aimed not only at indentifying the salinity effects on the species growth and productivity, but also at testing its crop potentiality at representative agricultural field conditions. Importantly, coastal environments seem ecologically optimal to support food production obtained by salt tolerant species irrigated with saline water. In fact, such soils are naturally exposed to saline conditions. Accordingly, seawater would not negatively affect the soil structure and fertility, which is generally very poor. Moreover, halophyte crop production in coastal areas would not compete with conventional crops production, as different resources (i.e. water and land) are used.

Beside the salt tolerance, several high quality food characteristics were evaluated as plants possible adaptation response to salinity. In fact, among the numerous biochemical strategies adopted by plants to cope with salinity, certain are linked to the hyperproduction of certain secondary metabolites, most of them representing high-nutrient compounds which are precious for human diet. To provide some examples, anthocyanins, polyphenols and proline content are known to increase accordingly with increasing salinity (Parida and Das, 2005). These compounds, referred also as plant stress metabolites, are produced by plants to interact with the environment in terms of adaptation and defense (Ramakrishna and Ravishankar, 2011), and are known to have important roles in human nutrition. Likewise, the selective accumulation or exclusion of ions caused by salinity (Parida and Das, 2005) also can reflect into interesting nutritional improvement achievable in salinity conditions. For example, calcium, among the main mineral elements lacking in the diet of over two-thirds of the world's population (White and Broadley 2009), is generally accumulated under saline conditions. Possible nutrient improvements caused by a saline environment are thus investigated. The contents of the different chapters are as follows:

Chapter 2.

In this chapter the possibility of growing three horticultural crops (i.e. lettuce, chard and chicory) with three seawater and freshwater blends in hydroponic conditions was explored. In particular, the crops were grown using 5%, 10% and 15% of seawater, corresponding to the following electrical conductivity (EC) values: 3dS m^{-1}, 6dS m^{-1}, 9dS m^{-1}. Crops growth, water consumptions and water use efficiency (WUE) were investigated to assess the maximum salinity tolerance thresholds of the different species. Photosynthetic parameters (i.e. photosynthetic activity and stomatal conductance) were evaluated too on a weekly basis to monitor the plants responses to the seawater irrigation. At the end of the trial, principal mineral elements, soluble sugars, carotenoids and phenolics concentration in the edible leaves were determined at the different salinity levels.

Chapter 3.

In this chapter different cropping systems for food production were compared on the basis of their need in water. In particular, chicory was the case study crop chosen. Its need in water during the whole production was compared among open field cultivation, conventional hydroponics and seawater hydroponics, using a share of 10% of seawater in its growing medium. The different water consumptions were compared by using the water footprint (WF) indicator, that indicates the overall amount of freshwater involved in the production cycle of any good or service.

Chapter 4.

In this chapter the effects of increasing levels of seawater irrigation were assessed on growth and productive performance of the salt-tolerant edible halophyte *Mesembryanthemum crystallinum*, the common ice plant, at representative agricultural conditions. In particular, six treatments were used in the experiment, each one repeated in three plots. Treatments were characterized by the following electrical conductivities (EC) values: 4dS m^{-1}, 8dS m^{-1}, 12dS m^{-1}, 16dS m^{-1}, 20dS m^{-1}, and 35dS m^{-1}. An additional control treatment (repeated in three plots as well) was characterized by EC of 2dS m^{-1}. Together with the ice plant crop potential, a full screening of morphological (i.e. specific leaf area, leaf succulence, leaf dry matter content and leaf water content) and physiological (i.e. chlorophyll concentration) characteristics was assessed to investigate eventual adaptations to salinity. The effects of different salinity levels on 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 were assessed too, i.e. mineral elements, carotenoids, soluble sugars, phenolic compounds and the antioxidant activity.

Chapter 5.

In this chapter the conclusions of the preceding chapters are synthesized and combined together. This chapter concludes with perspectives for future research on seawater use in agriculture.

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Chapter 2. Potential and constraints of different seawater and freshwater blends as growing media for three vegetable crops

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Chapter 2. Potential and constraints of different seawater and freshwater blends as growing media for three vegetable crops

Abstract

Alternative water sources for irrigation are needed to be found, as agriculture is currently using 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); 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 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, 1966). 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; Sgherri et al., 2008, 2007; Turhan et al., 2014), 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 (Ünlükara et al., 2008) or reduction (Bartha et al., 2015; Kim et al., 2008; Turhan et al., 2014) 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.

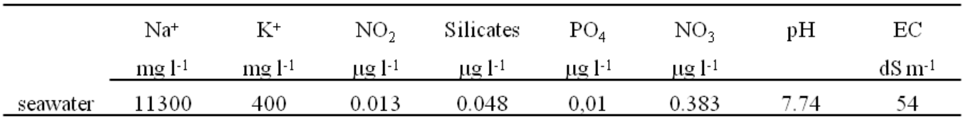
2. Materials and methods

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 5cm 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 (2015) - 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 µmol 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 minutes 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 20L 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™).

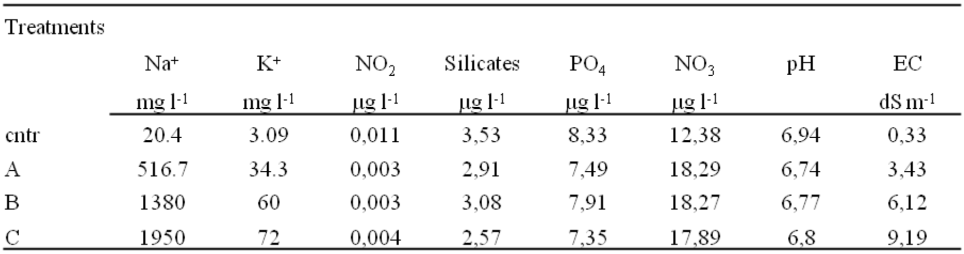
Table 1: Seawater chemical and physical characteristics



	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	11300	400	0.013	0.048	0,01	0.383	7.74	54

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 dSm^{-1}; A: 5% seawater EC=3.4 dSm^{-1}; B: 10% seawater EC=6.1 dSm^{-1}; C: 15% seawater EC=9.2 dSm^{-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 2: Growing media chemical and physical characteristics: average values of the three species measurements



Treatments	Na^+	K^+	NO_2	Silicates	PO_4	NO_3	pH	EC
	mg l^{-1}	mg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}		dS m^{-1}
cntr	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 and WUE. 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 thanks 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 and as the ratio between the fresh marketable biomass (yield) and total ET throughout the crop cycle, as follows:

$$WUE_{wp} = \text{whole plant dry biomass (g) / ET (L)}$$

$$WUE_{y} = \text{fresh marketable biomass (g) / ET (L)}$$

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 0.5mg 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 100mg 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 25ml 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-visible spectrophotometer (Bio-Rad SmartSpec^{TM}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 100mg/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 µl of the Folin-Ciocalteu reagent were added to 125 µl of the suitably diluted sample extract. After 6 minutes, 1.25 ml of a 7% aqueous Na_2CO_3 solution was added. The final volume was adjusted to 3 ml. After 90 minutes, 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 to assess significant differences between treatments. Significance level was P ≤ 0.05 (unless differently stated).

3. Results and discussion

3.1 *Growth, biomass yield and WUE*

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 (Figure 1).

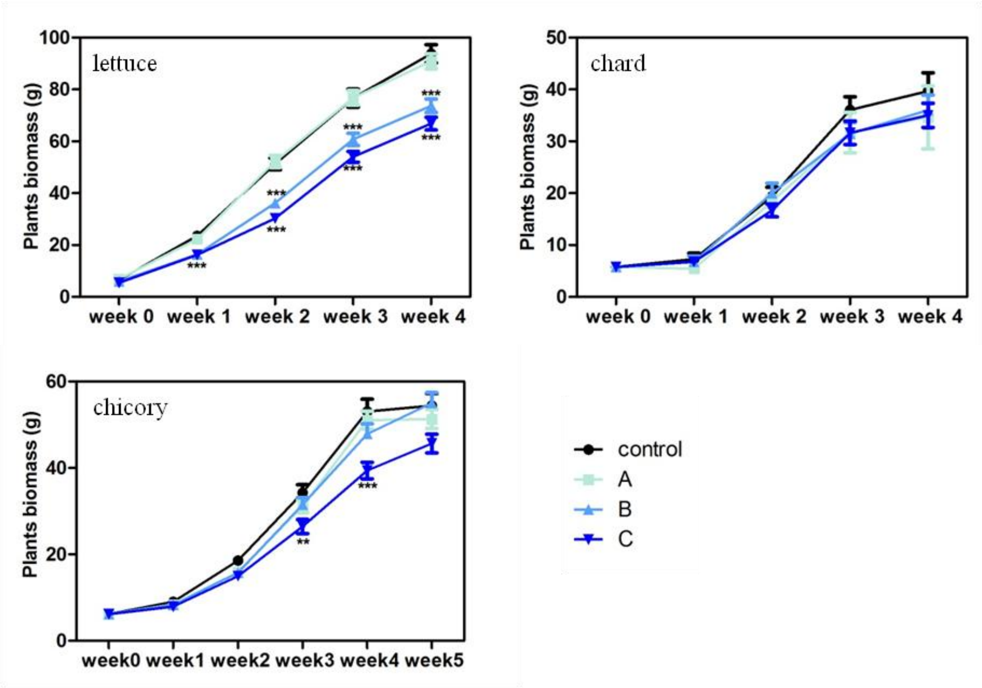
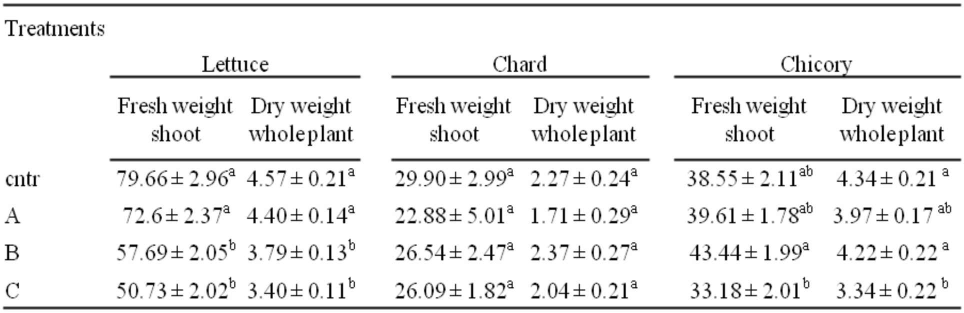


Figure 1: Lettuce, chard and chicory biomass production: values are means of the entire plant fresh weight ± SEM. Asterisks indicate significant difference from control (* at $P < 0.05$; ** at $P < 0.01$, *** at $P < 0.0001$ Tukey's Test)

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).

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



Treatments	Lettuce		Chard		Chicory	
	Fresh weight shoot	Dry weight wholeplant	Fresh weight shoot	Dry weight wholeplant	Fresh weight shoot	Dry weight wholeplant
cntr	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}

Chard biomass did not show any significant difference among the four treatments and during the whole crop cycle (Figure 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 (Figure 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.

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 (Figure 2).

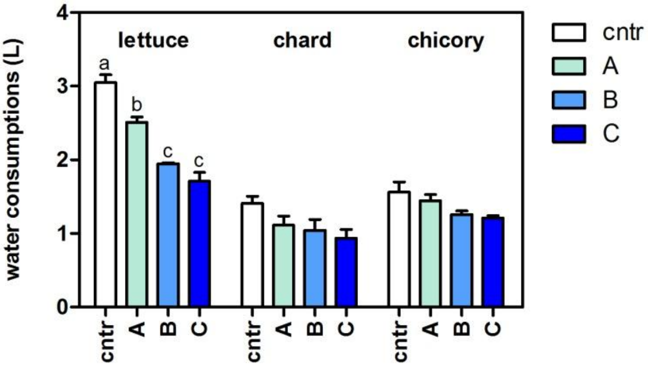


Figure 2: Plant water consumption. Values are means ± SEM expressed in liters per plant. Different letters indicate a significant difference at $P < 0.05$ (Tukey's Test)

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 (Figure 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 WUE 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 WUE for biomass production.

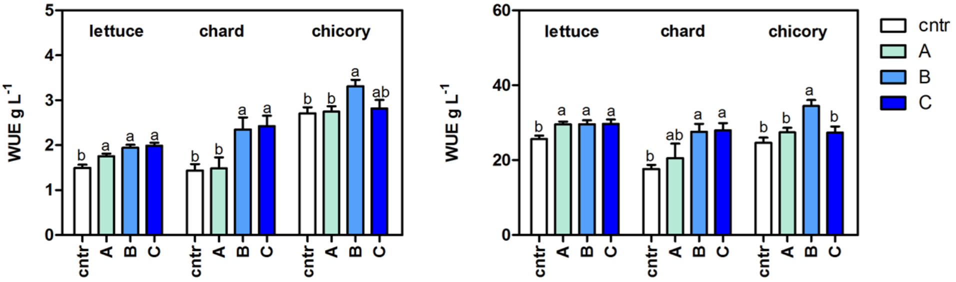


Figure 3: WUE as a ratio between the plant total dry biomass and tot ET (WUE_{wp}, graph 1); WUE as a ratio between the fresh marketable biomass and tot ET (WUE_y, graph 2). Values are means ± SEM expressed in gram per liter. Different letters indicate a significant difference at $P < 0.05$ (Tukey's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

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 (Figure 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 (Figure 5). Similarly, significant differences in A_n between control and treated plants were found in chard (Figure 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 (Figure 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 (Figure 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 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.

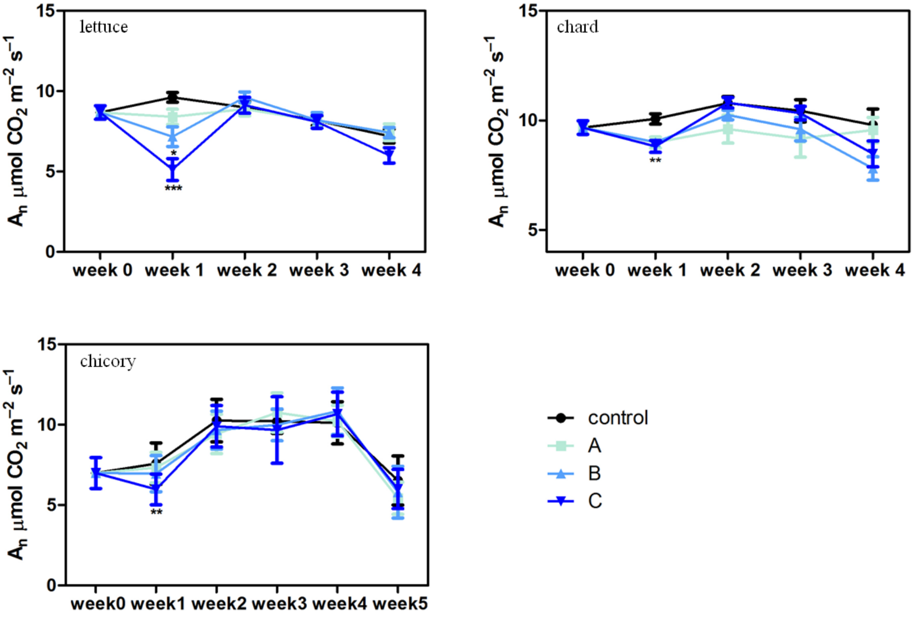


Figure 4: Lettuce, chard and chicory A_n during the crop cycle: values are means ± SEM. Asterisks indicate significant difference from control (* at $P < 0.05$; ** at $P < 0.01$, *** at $P < 0.0001$ Tukey's Test)

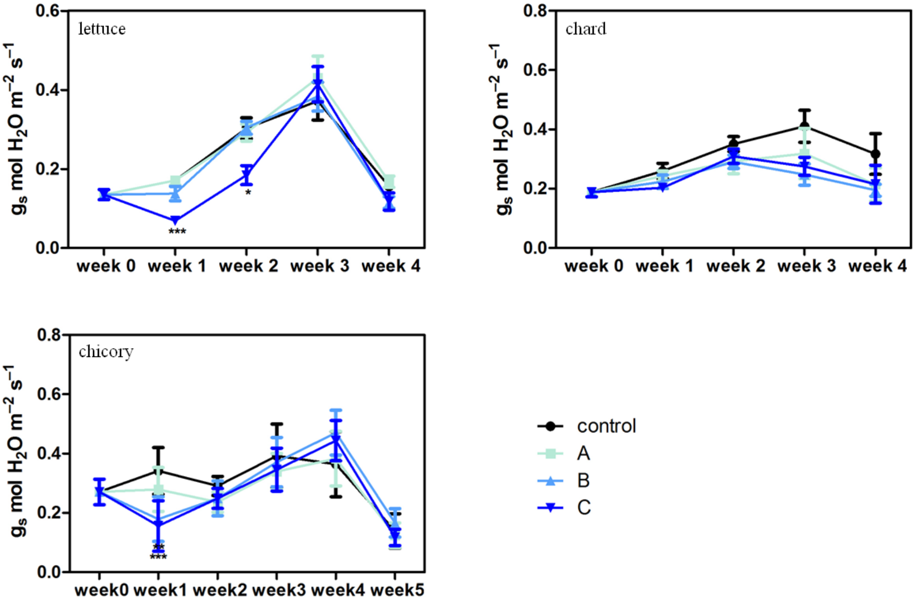
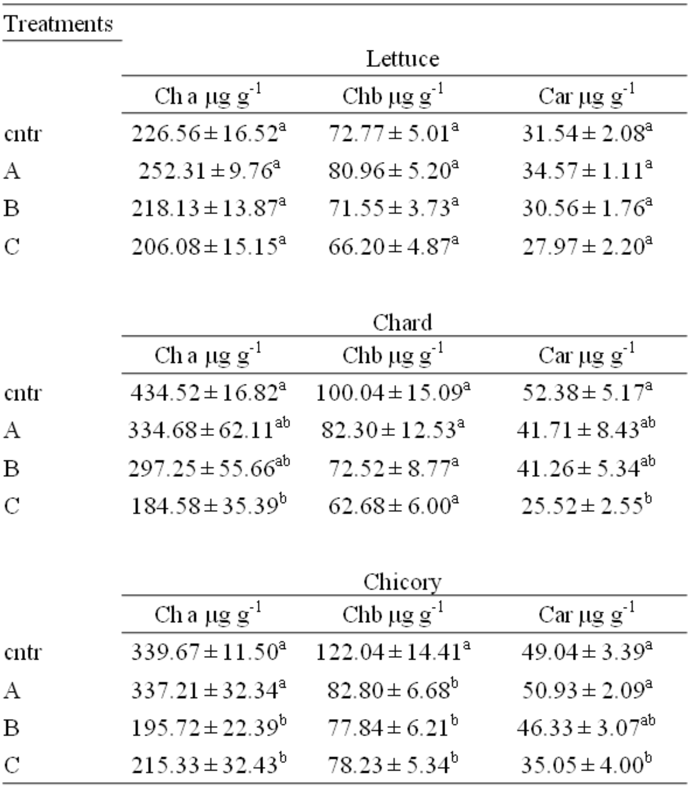


Figure 5: Lettuce, chard and chicory g_s during the crop cycle: values are means ± SEM. Asterisks indicate significant difference from control (* at $P < 0.05$; ** at $P < 0.01$, *** at $P < 0.0001$ Tukey's Test)

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

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)



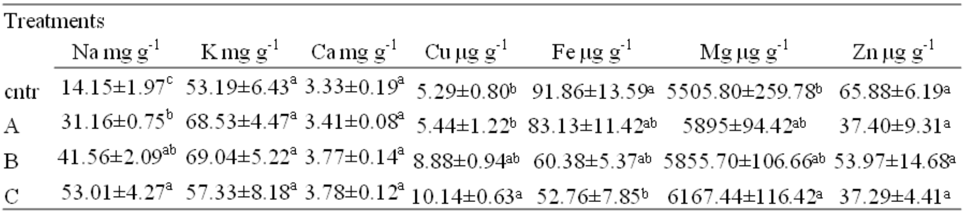
Treatments			
	Lettuce		
	Ch a $\mu g\ g^{-1}$	Chb $\mu g\ g^{-1}$	Car $\mu g\ g^{-1}$
cntr	226.56 ± 16.52^{a}	72.77 ± 5.01^{a}	31.54 ± 2.08^{a}
A	252.31 ± 9.76^{a}	80.96 ± 5.20^{a}	34.57 ± 1.11^{a}
B	218.13 ± 13.87^{a}	71.55 ± 3.73^{a}	30.56 ± 1.76^{a}
C	206.08 ± 15.15^{a}	66.20 ± 4.87^{a}	27.97 ± 2.20^{a}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

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

Tables 5, 6 and 7 report 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 Valente 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).

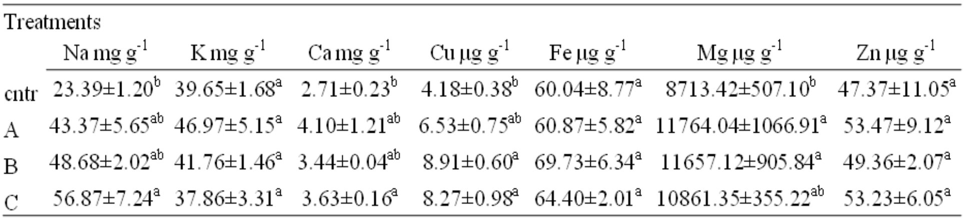
Table 5: Elements accumulation in lettuce leaves. Values are means ± s.e. expressed per gram of leaves dry weight. Different letters in the same column indicate a significant difference at $P < 0.05$ (Tukey’s Test)



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}
cntr	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}

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).

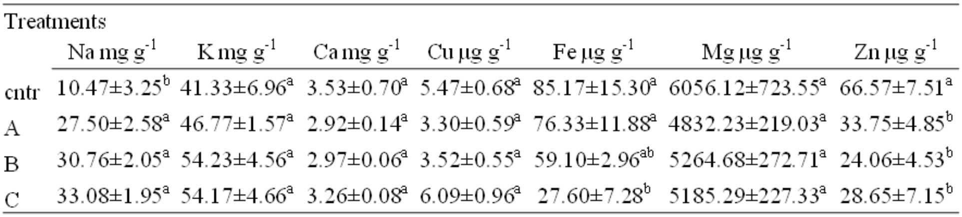
Table 6: Elements accumulation in chard leaves. Values are means ± s.e. expressed per gram of leaves dry weight. Different letters in the same column indicate a significant difference at $P < 0.05$ (Tukey’s Test)



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}
cntr	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}

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).

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}
cntr	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}

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, Ünlükara et al. (2008) showed no significant differences in K^+ accumulation between different salinity levels (up to 7.0dS 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 (Lazof and Cheeseman, 1988; Ünlükara et al., 2008). 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 (Al‐Karaki, 2000; Lutts et al., 1996; Ünlükara et al., 2008; Yousif et al., 2010).

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 (Gao et al., 1998; Naeini et al., 2004; Turhan et al., 2014). In particular, 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).

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}
cntr	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}

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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Chapter 3. Water footprint comparison among different cropping systems: a chicory (*Cichorium intybus* L.) case study

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Chapter 3. Water footprint comparison among different cropping systems: a chicory (*Cichorium intybus* L.) case study

Abstract

Food security is strongly linked with water. Many indicators take into account water use in food production. The water footprint (WF), among them, indicates the freshwater amount involved in the production cycle. This study examines chicory crop WF in different cropping systems: open-field cultivation, conventional hydroponics and seawater hydroponics (i.e. with 10% seawater in the nutrient solution). WF in hydroponics and sea-hydroponics were significant lower (i.e. 63.9% and 74.5%) compared to the open-field cultivation. The WF of chicory in sea-hydroponics was 29.5% lower than in conventional hydroponics. These results open new avenues for seawater-based chicory horticulture in the upcoming years.

1. Introduction

Food security, intended as the ability to meet the energy needs of the world population, is strongly linked with water use (FAO, 2011). To ensure an adequate food production to the increasing world population, reaching up to 10.9 billion people by the end of the century (UN, 2013), the human pressure on water resources will increase. Many indicators take into account water use in food production: the water footprint (WF), among the others, indicates the total volume of freshwater appropriated during production, considering the volumes of water consumed and polluted in the different steps of any food production cycle (Hoekstra et al., 2011). According to FAO, an average of 1.000 - 2.000 and of 13.000 - 15.000 liters of freshwater is required to produce one kilogram of wheat and meat respectively (Mekonnen & Hoekstra 2010). Besides, freshwater availability is already an issue in many regions, because the agricultural sector uses nowadays about 70% of the world stock. Nevertheless, to guarantee the world population's food security, actions should be taken both improving the water efficiency of cropping systems and seeking alternative water sources in agriculture.

Water efficiency improvements in agriculture have been in the spotlight for many years and consistent advances have been already made. Soilless culture, intended as any method of growing plants without the use of soil as a rooting medium, in which nutrients are supplied via the irrigation water (FAO, 2013), is characterized by a particular attention to the water resource. Soilless culture systems involve different techniques, the plant roots growing either in porous substrates or directly in nutrient solution with no solid phase, in open or close-cycle cultivation systems, this last ones also reusing the drainage solution (FAO, 2013). Among the different soilless systems, hydroponics is characterized by an expanding worldwide vegetable production of about 35000 ha (Hickman, 2011). Although this technique has been used on a commercial basis for only 50 years, it has already been adapted to many situations: from innovative/futuristic conditions to developing countries, where it can provide intensive food production (Sheikh, 2006). Interestingly, hydroponics allows the saving of about the 10-30% of water compared to soil cultivation, also reducing the use of fertilizers and pesticides (Sheikh, 2006).

Despite those remarkable achievements, even more can be obtained in terms of freshwater saving by combining innovative growing systems and alternative water sources, i.e. seawater. In fact, seawater represents the most abundant source of water on earth (FAO, 1993) and because it is rich of most plant nutrients (Eyster, 1968) can be regarded as a realistic option in agriculture, either desalinized or blended with freshwater (Yermiyahu et al., 2007). Thus, the use of seawater in soilless agricultural systems, without negative salt input in soils, would ensure a twofold goal: to decrease the freshwater demand while exploiting the seawater nutrient content.

The current study investigated the WF of chicory (*Cichorium intybus* L.) grown with three different techniques: conventional field cultivation, conventional NFT (nutrient film technique) hydroponics, NFT hydroponics with a share of the 10% of seawater blended with the nutrient solution (sea-hydroponics).

2. Materials and methods

2.1 Open field cultivation water footprint estimation

Chicory's evapotranspiration (ET) in open field cultivation was calculated using the Food and Agriculture Organization CROPWAT model (FAO, 2010), following the guidelines reported by Hoekstra *et al*. (2011). Between the two options for calculating crops evapotranspiration, the "irrigation schedule option" was chosen because of its higher accuracy. Climate data were obtained from FAO's software CLIMWAT 2.0 for CROPWAT related to the following parameters: location 23, Florence, altitude 51m, 43.76°N, 11.25°E. Both location and crop planting date parameters were chosen to correlate the model results with empirically obtained data.

According to "The Water Footprint Assessment Manual" (Hoekstra et al., 2011), the crop water footprint (WF) was calculated as follows:

$$WF = (ET * 10)/Y$$

Yield (Y) data were obtained from the FAOSTAT Database.

2.2 Hydroponics and sea-hydroponics water footprint assessment

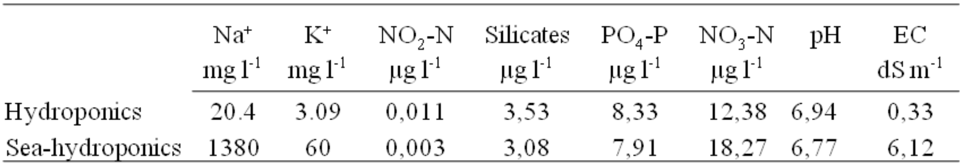
Hydroponics and sea-hydroponics water footprint were empirically calculated. Chicory crop was grown in 2014 at the greenhouse facilities at the University of Florence, Italy, as reported in Atzori et al. (2016). Ten-day-old seedlings were bought at a nursery and grown for an additional 10 days in hydroponics with a nutrient solution made of tap water and liquid fertilizer Flora Series™ (General Hydroponics Europe Inc). Afterwards, plants were transferred in a closed-cycle NFT hydroponic system, from September the 17th to October the 30th. Of six independent hydroponic lines, each one bearing 9 plants, three contained tap water and nutrient solution (hydroponics: electrical conductivity (EC) = 0.3 dS m^{-1}), whereas three contained tap water, nutrient solution and 10% of seawater (sea-hydroponics: EC = 6.1 dS m^{-1}). Seawater used in this trial was collected one week before the beginning of the experiment at Marina di Pisa (Italy), then stored in 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-P, NO_3–N 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™).

Table 1: Seawater chemical and physical characteristics

	Na^+	K^+	NO_2	Silicates	PO_4-P	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}
seawater	11300	400	0.013	0.048	0,01	0.383	7.74	54

Throughout the trial, pH and EC were measured twice a week, while the growing media were replaced every two weeks (their chemical and physical characteristics are reported in Table 2). Plants were maintained in normal greenhouse humidity (relative humidity: 40-55%) and without artificial light, light intensity reaching 700 µmol m^{-2} s^{-1} during sunny days, and at 23°C/13°C day/night temperature.

Table 2: Growing media chemical and physical characteristics



	Na^+ mg l^{-1}	K^+ mg l^{-1}	NO_2-N µg l^{-1}	Silicates µg l^{-1}	PO_4-P µg l^{-1}	NO_3-N µg l^{-1}	pH	EC dS m^{-1}
Hydroponics	20.4	3.09	0,011	3,53	8,33	12,38	6,94	0,33
Sea-hydroponics	1380	60	0,003	3,08	7,91	18,27	6,77	6,12

Plants biomass weight was recorded at the end of the experiment. In particular, fresh shoot weights were collected, corresponding to the edible part of this species, thus to yield. Crop evapotraspiration (ET) was recorded on a weekly basis by measuring the volume of solutions consumed by plants of each treatment: the thanks containing the recirculating growing media had liter graduations, thus allowing the recording of plants water consumption (assuming zero water loss apart from plant evapotraspiration, being our experimental set up a closed-cycle hydroponic system). After assessing that biomass production was not negatively affected by the seawater in the growing media (Atzori et al., 2016 and Chapter 2), water footprint (WF) was calculated as the ratio between the total evapotranspiration during the crop cycle and the fresh shoot biomass at harvest time.

2.3 Statistical analysis

The experimental set-up related to the hydroponics and sea-hydroponics water footprint assessment was randomized to guarantee uniform conditions between the two treatments. Two statistical analyses were conducted using GraphPad Prism for Windows. One-way ANOVA with Dunnett post-test analysis was used to compare both hydroponics and sea-hydroponics water footprint to the open field simulated water footprint, considered as the reference value (significant differences at $P < 0.0001$). Along with this, to assess significant differences between the two soilless systems, hydroponics and sea-hydroponics water footprint values were compared with a t test ($P < 0.0001$).

3. Results and discussion

The water footprint values obtained with CROPWAT model and with the two hydroponic systems are reported in Figure 1.

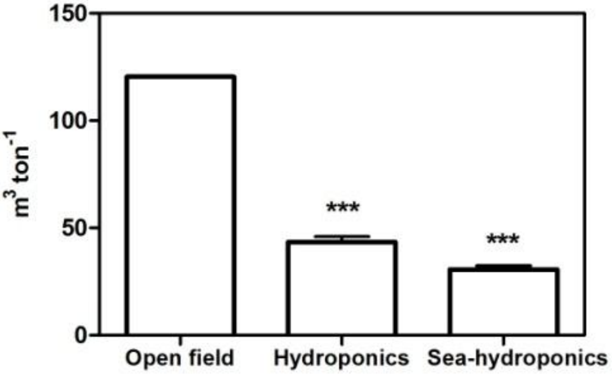
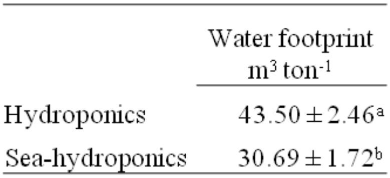


Figure 1. Water footprint values of chicory in three different growing systems. Values of hydroponics and sea-hydroponics are means ± SEM. Asterisks indicate significant difference compared to the open field value at $P < 0.0001$ (Dunnett Test).

Significant differences were observed between each soilless system compared to open field cultivation, assessing for hydroponics and for sea-hydroponics water footprint reduction of 63.9% and of 74.5%, respectively.

As reported in Table 3, the T test assessed significant differences between the two soilless systems too, with the sea-hydroponics achieving a water footprint reduction of 29.5% compared to conventional hydroponics.

Table 3: Water footprint values of chicory in hydroponics and sea-hydroponics. Values are means ± SEM. Different letters indicate a significant difference at $P < 0.0001$ (T Test).



	Water footprint m^3 ton^{-1}
Hydroponics	43.50 ± 2.46^{a}
Sea-hydroponics	30.69 ± 1.72^{b}

Open field results are consistent with those reported by Mekonnen and Hoekstra (2010) related to chicory production in the Tuscany region. Likewise, results obtained on hydroponic cultivation confirm the increased water use efficiency of hydroponics growing systems. Interestingly, sea-hydroponics assessed the best performances in water saving, not at a cost of biomass production, which in fact was not negatively affected by the share of seawater added to growing media (complete data on chicory biomass production are reported in Atzori et al. 2016 and in Chapter 2). This result is particularly important because the best opportunity for water efficiency enhancement is to close the gap between yield and water use performance (Mateos and Araus, 2016). In fact, even if 10% of seawater may represent a limited share of the solution needed by the crop, on a large scale hydroponics production this same percentage becomes remarkable. Moreover, several other crops might be grown with an even higher share of seawater, not resulting in negative yield productions though limiting freshwater use. Interestingly, seawater in the growing media implies, for certain crops, the nutritive improvement of the edible product (Atzori et al., 2016; Sgherri et al., 2008).

Although further investigations comparing the water footprint and the carbon footprint of the here tested growing systems might be interesting to analyze the growing systems efficiency and its costs on the environment from a comprehensive perspective, these results suggest a realistic advantage in saving water without productive disadvantages.

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Chapter 4. Effects of increased seawater salinity irrigation on growth and quality of the edible halophyte *Mesembryanthemum crystallinum* L. under field conditions

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Chapter 4. Effects of increased seawater salinity irrigation on growth and quality of the edible halophyte *Mesembryanthemum crystallinum* L. under field conditions

Abstract

Saline agriculture may answer to the declining availability of fresh water and to the worldwide expanding area of salinized soils by exploiting seawater and salt-affected soils for sustainable food production. Potential salt tolerant crops can be found among edible halophytes. Plants growing in saline environments are often associated with an enhanced endogenous concentrations of high-nutrient compounds. *Mesembryanthemum crystallinum* L. provides an interesting perspective in becoming a salt-tolerant and high-value crop at saline conditions, but has never been tested at representative agricultural conditions. This study aimed at assessing the effects of increasing levels of seawater salinity irrigation (EC: 2, 4, 8, 12, 16, 20, and 35 dS m^{-1}) on growth in a field experiment. Also, impacts of salinity on the functional value of edible leaves were evaluated by investigating the mineral elements, carotenoids, soluble sugars, phenolic concentrations, antioxidant activity. None of the salinity treatments negatively affected *M. crystallinum* biomass production. Furthermore, increased salinity extended the vegetative stage, leading to one extra month of harvest compared to non-saline conditions. Juvenile edible leaves' biomass, succulence and calcium concentrations even increased with increasing salinity. No differences were assessed in the phenolics concentration and antioxidant activity of high salinity treatments plants compared to the control. This paper demonstrates the perspective to cultivate *M. crystallinum* in saline agriculture, up to EC of 20-35 dS m^{-1}, or perhaps even higher, since we did not identify a threshold of biomass reduction. Only the Na^+ concentration in the edible leaves could constitute a health concern or allow it acting as a natural salt substitute. This excellent performance in combination with the appreciated taste

and its glistening appearance may pave the way for use of the ice plant as high-value saline crop.

1. Introduction

Because of the growing population, the human pressure on global water resources will tremendously increase. Moreover, as a result of global warming, increasing saline and dry conditions may affect arable land and sea level rise is becoming an issue in coastal areas. Many conventional crops are salt sensitive although some may be grown up to 30% seawater salinity (Koyro et al., 2011). Above this salt concentration, major yield reductions are observed, due to water stress, ion toxicity, nutritional disorders, oxidative stress, metabolic processes alterations, membrane disorganization and genotoxicity among other factors (Munns, 2002). Therefore, saline agriculture, working with salt-tolerant crops, may represent an answer to the declining availability of fresh water and to the worldwide expanding area of salinized soils. Salt tolerant species allow exploitation of the great availability of brackish and sea water, making coastal and salt affected areas productive.

Salt-tolerant species - halophytes - native from salt marshes and inland saline sites, can grow and reproduce on saline soils on which 99% of the other species get deprived (Flowers and Colmer 2008). Their adaptation to saline environments may be characterized as salt tolerance or as salt avoidance (Koyro et al., 2011). A wide range of morphological, physiological, and biochemical adaptations exists in halophytes, varying widely in their degree of salt tolerance (Flowers and Colmer 2008). In some cases, salinity may even result in favorable effects for yield and its quality (Flowers and Muscolo, 2015; Shannon and Grieve, 1998). Although halophytes represent just 2% of the terrestrial plant species, they are represented in half of the higher plants' families (Glenn et al., 1999). Because many of those salt tolerant plants are edible, their domestication and cultivation in a saline agriculture context could be regarded as an interesting approach to consider (Glenn et al., 1998; Rozema and Flowers, 2008; Rozema and Schat, 2013; Ventura et al., 2015).

Saline agriculture may pursue relevant goals. To begin with, an increased sustainable food production could be achieved exploiting resources which would not be used for conventional agriculture: seawater or brackish waters as complementary irrigation waters and salt-affected soils (Glenn et al., 1999). Importantly, agricultural areas lost because of salinization would be regained for staple food production (Bruning and Rozema, 2013). The numerous biochemical strategies adopted by plants to cope with salinity include the selective accumulation or exclusion of ions; the synthesis of osmotic solutes; the induction of antioxidant compounds (Parida and Das 2005), or secondary metabolites (Ramakrishna and Ravishankar, 2011). The high endogenous concentration of such compounds, known to have healthy properties for human consumption, can be precious for the nutritive characteristics of food (Di Baccio et al., 2004; Sgherri et al., 2008). Thus, halophytes grown in saline conditions could also be a source of compounds with a potential added nutritive and economic value (Flowers and Muscolo 2015), representing high-value crops.

Mesembryanthemum crystallinum L., the common ice plant, Aizoaceae, Caryophyllales, is a succulent, prostrate annual herb with high potential for becoming a salt-tolerant high-value crop. Native to southern and eastern Africa, and nowadays widespread along the coastal areas of Europe, USA, Mexico, Chile, the Caribbean and western Australia (Adams et al., 1998), this species is already being consumed as a vegetable crop in several countries such as India, California, Australia and new Zealand and in some countries of Europe (Agarie et al., 2009), i.e. in Germany (Herppich et al., 2008) and in The Netherlands. It has been known as a traditional medicine as well, in particular for its demulcent and diuretic effects (Bouftira et al. 2012), a relevant content of superoxide dismutase (SOD) and related anti-oxidants molecules, which have a role in the protection of the skin against radiation exposure (Bouftira et al., 2008), and for its antiseptic properties (Ksouri et al., 2008). The ice plant main particularity is that its entire above ground surface is covered with unicellular trichomes, ranging from 1 to 3 mm in diameter

(Vivrette and Muller, 1977), called bladder cells, filled with a water solution and functioning as peripheral salinity and water reservoirs providing protection from short term high salinity or water deficit stress (Agarie et al., 2007; Luttge et al., 1978). Interestingly, its morphological adaptations, together with the capability to switch from C_3 photosynthesis to Crassulacean acid metabolism (CAM), make this species able to complete its life cycle on soil containing NaCl at a concentration comparable to seawater (Adams et al., 1998).

Because of its considerable resistance to salt and drought stress (Bloom, 1979; Vivrette and Muller, 1977), the ice plant has been studied as a model species starting from the 80s (Bohnert et al. 1988). From then onwards, a number of laboratory experiments aiming at elucidating the physiological and molecular mechanisms behind its stress resistance have been published, and among the many studies we recall the work of Agarie et al., 2007; Barker et al., 2004; Cosentino et al., 2010; Kore-eda et al., 2004; Oh et al., 2015; Sanada et al., 1995; Thomas et al., 1992; Thomas and Bohnert, 1993; Vernon and Bohnert, 1992; Winter and Holtum, 2007. Yet, despite the physiological understanding, scientific documentation at field conditions is very scarce. Hence, there is a strong need for large-scale field experiments to evaluate the feasibility of *Mesembryanthemum crystallinum* for halophyte crop production, together with the economic perspective for future growers (Ventura et al., 2015). Such studies - ideally executed at multiple salinity levels - are essential for verifying the crop potential of this species in saline soils and/or to estimate the production of specific osmotic solutes and secondary metabolites, known to have multiple healthy properties for humans, synthesized by the plant as protection against oxidative effects. In particular, the effect of different salinity levels on such production in the ice plant has never been investigated.

The present study had thus a twofold aim: *i*) to evaluate the crop potential of *Mesembryanthemum crystallinum*, by determining the effects of seawater irrigation on growth and productive performances in a field experiment with a wide range of salinity conditions in an agricultural setting. A full screening of morphological (i.e. specific leaf area, leaf succulence, leaf dry matter content and leaf water content) and physiological (i.e. chlorophyll concentration) characteristics was conducted to investigate eventual adaptations to salinity. *ii*) to assess the effects of different salinity levels on 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, i.e. mineral elements, carotenoids, soluble sugars, phenolic compounds and the antioxidant activity.

2. Materials and methods

2.1 Research location, irrigation and soil salinity

Mesambryanthemum crystallinum was grown at increasing salinity levels in an experimental field on Texel island (53.012837°N, 4.755306°E), The Netherlands, from May to August 2015. As described in detail by Bruning and collaborators (2015), the experimental area consisted of a field divided into 21 plots (8 × 20 m each) with seven salt concentrations randomly distributed and replicated three times. Three years prior to the experiment, the soil, which mainly consists of sand (3% loam, 2% clay, and 2% organic matter), was homogenized by a large power shovel mixing the top first meter of soil for 3 days. The plots were drip irrigated daily with 12 mm day^{-1} so that the soil moisture never dropped below the 80% of the soil water-holding capacity. Individual drip lines were located at 40 cm intervals, with drippers each 30 cm distance. The irrigation water was a mixture of fresh water from a rainwater basin and natural seawater from a ditch fed from the Waddensea (electrical conductivity EC of 35 dS m^{-1}). A custom-built proportional-integral-derivative (PID) controller mixed fresh and saline waters with frequency-regulated pumps from both water sources, which allowed time-based automatic pulse irrigation with an automatic accuracy check of salinity levels in the irrigation water. Drainage pipes, located 60 cm below the surface with 5m spacing between any two pipes, assured a rapid drainage of the daily irrigation water and aeration of the soil.

The six treatments used in the experiment, each one repeated in three plots, were characterized by the following electrical conductivities (EC) values: 4dS m^{-1}, 8dS m^{-1}, 12dS m^{-1}, 16dS m^{-1}, 20dS m^{-1}, and 35dS m^{-1}, plots salinity gradually reaching the target values within the half of June. An additional control treatment (repeated in three plots as well) was characterized by EC of 2dS m^{-1}. Soil salinity was monitored during the experiment by means of soil pore water samples, collected in all plots three times during the experiment. The ECe was approximated from the EC of soil pore water obtained by means of suction cups that were placed at 0–10 cm, 20–30 cm and 50–60 cm depth in all the plots, as a strong correlation was found between EC_{pw} and EC_e ($EC_e = 0.69 \times EC_{pw}$, with $r^2 = 0.82$). Such EC values did not deviate from the targeted EC of the treatments.

2.2 Plant material, sampling and growth measurements

Seeds of *Mesambryanthemum crystallinum* were sown on the 14th of April 2015 and young seedlings were transferred after one month into the experimental fields with 30 plants per plot. Three sampling events were performed during the experiment: *i*) time zero sampling (T0), on 6 untreated juvenile plants 5 weeks from germination old; *ii*) as plants' young fully expanded leaves were ready to be harvested, thus at the potential commercial maturity (T1, 16th of July 2015, on three plants per plot, nine per treatment); *iii*) at the end of the crop cycle (T2, 11th of August 2015, on three plants per plot, nine per treatment), with some plants beginning seed production/drying up.

At the T0 sampling, the total fresh and dry shoot weight was collected ($n = 6$). Dry material was then used to assess the Na^+, K^+ and Ca^{2+} concentration in juvenile plants. At T1 and T2, the total shoot fresh weight and the fresh weight of three young fully expanded leaves per plant were recorded. Leaf area (LA) of young fully expanded leaves was calculated using ImageJ software ($n = 9$). Specific leaf area (SLA), leaf succulence, leaf dry matter content (LDMC) and leaf water content (LWC) were determined on young fully expanded leaves ($n = 9$) to investigate possible morphological adaptations to salinity. Samples of young fully expanded leaves (*n* = 9) were collected, fresh weighed, frozen in liquid nitrogen and stored at -20˚C for further analysis of soluble sugar, chlorophyll, carotenoids and phenolics concentration. Plant shoots were oven-dried (70˚C until constant weight) and total shoot dry biomass and young fully expanded leaves dry weight were determined. Dried samples from young fully expanded leaves were then used for measuring the Na^+, K^+ and Ca^{2+} concentration and the antioxidant activity.

The specific leaf area (SLA), leaf succulence, leaf dry matter content (LDMC) and leaf water content (LWC) were determined as follows:

$$SLA = L_A/L_{DW}$$

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

$$\text{leaf succulence} = L_{FW}/L_A$$

with L_{FW} = leaf fresh weight (g) and L_A = leaf area (cm^2), often used as an estimate of leaf succulence (Agarie et al., 2007; Jennings, 1976)

$$LDMC = L_{DW}/L_{FW}$$

with L_{DW} = leaf dry weight (g) and L_{FW} = leaf fresh weight (g) (Garnier et al., 2001)

$$LWC = (L_{FW} - L_{DW})/L_{FW}$$

with L_{FW} = leaf fresh weight (g) and L_{DW} = leaf dry weight (g), according to the commonly used formula.

2.3 Measurement of chlorophyll and carotenoid concentrations

Total leaf chlorophyll and carotenoid concentrations were determined in young fully expanded leaves (T1 and T2, *n* = 9). Cold 100% methanol was added to the frozen grounded tissues and samples were left shaking in darkness at 4°C for 30 minutes to extract pigments. After that, samples were centrifuged at 1000 rpm for 10 minutes. The supernatant was collected and used to read the absorbance at 665, 652 and 470 nm using a Tecan Infinite 200 Spectrophotometer (Männedorf, Switzerland). Chlorophyll a, chlorophyll b and carotenoid concentrations were determined according to Wellburn (1994).

2.4 Measurement of Na^+, K^+, and Ca^{2+} concentrations

The Na^+, K^+ and Ca^{2+} concentration in young fully expanded leaves (T0: *n* = 6; T1 and T2: *n* = 9) was obtained after digesting ground dried tissues in 0.5 M HNO_3 by shaking vials in the dark at 25 °C for 48 h, as in Bazihizina et al. (2015). Diluted extracts were analyzed using a Flame Photometer Digiflame2000 (Lab Services SAS, Rome, Italy). The values of the calibration curve ranged from 0 to 0.1 mg/ml for Na^+ and K^+ ($R^2 = 0.998$) and from 0 to 0.05 mg/ml for Ca^{2+} ($R^2 = 0.999$) determination.

2.5 Measurement of soluble sugars

The extraction of soluble sugars from young fully expanded leaves (T1 and T2, *n* = 9) was performed on frozen grounded samples by incubating samples with 80% ethanol in a 80°C water bath and centrifuged, this procedure being repeated twice to extract all soluble sugars. The supernatant was 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 samples at 620 nm in a UV-visible spectrophotometer (Bio-Rad SmartSpec^{TM}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 ethanol only and to extra tissue samples immediately prior to extraction. The values of the calibration curve ranged from 0 to 100 mg/ml of glucose ($R^2 = 0.997$).

2.6 Measurement of phenolics content and antioxidant activity

The total phenolic concentration (T1 and T2, *n* = 3) was measured using the Folin-Ciocalteu method, according to Dewanto et al. (2002). Deionised water (0.5 ml) and 125 µl of the Folin-Ciocalteu reagent was added to 125 µl of a suitably diluted sample extract. The mixture was allowed to stand for 6 min and then 1.25 ml of a 7% aqueous Na_2CO_3 solution were added. The final volume was adjusted to 3 ml. After 90 minutes, the absorption was measured at 760 nm against water as a blank using a Agilent 8453UV-Vis Spectrophotometer (Agilent Technology, Palo Alto, CA, USA). The amount of total phenolics is expressed as gallic acid equivalents (GAE, mg gallic acid/g sample) by using the calibration curve of gallic acid. The calibration curve ranged from 20 to 500 mg/ml ($R^2 = 0.997$).

The antioxidant activity (T1 and T2, *n* = 3) was measured with the 1,1-diphenyl- 2-picrylhydrazyl free radical (DPPH) assay on young fully expanded leaf samples of plants treated at EC of 12, 20, 35 dS m^{-1} and control. The DPPH quenching ability of plants extracts was measured according to Hatano et al. (1988): the amount of 0.5 ml of the extract at 5 different concentrations was added to 0.5 ml of a DPPH ethanolic solution. The mixture was shaken vigorously and the absorbance was read at 517 nm in a UV-visible spectrophotometer (Bio-Rad SmartSpec^{TM}Plus), at the very beginning and after 20 minutes from the beginning of the reaction. The scavenging activity on the DPPH radical was calculated as follows:

$$\text{DPPH scavenging effect (\%)} = [\,(A_0 - A_{20}) / A_0] \times 100$$

where A_0 is the absorbance at the beginning of the reaction and A_{20} is the absorbance after 20 minutes from A_0. The antiradical activity was expressed as EC50 (mg/mg), the antiradical dose required to cause a 50% inhibition. Lower EC50 corresponds to a higher antioxidant activity of the plant extract.

2.7 Statistical analyses

Statistical analyses were conducted using GraphPad Prism 5 for Windows. One-way analysis of variance was used to assess significant differences among treatments. In particular, the Tukey's Test was chosen to enable comparisons not only between salinity and control conditions, but also among all salinity levels. Significance level was $P \leq 0.05$ or higher, as reported in Tables and Figures captions.

3. Results

Growth. Figure 1 reports pictures of the control and of the higher salinity treatment plants at the commercial maturity time in July: the main observable difference between the two groups is the reddish color of salt-treated plants leaves, whereas plants were comparable in size suggesting that salt did not have any negative effect on plants growth. Moreover, control plants started shrinking/going to the seed production phase in August, while 35 dS m^{-1} treated plants were still maintaining the old larger leaves. In fact, the plant cycle lasted about one more month longer at the higher salinity treatments (i.e. 20 and 35 dS m^{-1}) compared to control conditions (authors field observations).

Figure 1: Photographs of the control and of the higher salinity treatment plants at the commercial maturity time (T1).

Results of total shoot fresh weight (FW) and dry weight (DW) during the experimental period are shown in Figure 2. No significant differences among treatments were assessed at T0, when the salinity treatments had not yet started, nor at T1, plants of all treatments reaching a comparable shoot biomass production. On the contrary, at T2, plants growing at higher salinity tended to have an increasingly higher biomass. Plants of 20 dS m^{-1} treatment showed a significant increase of both fresh and dry shoot biomass compared to control.

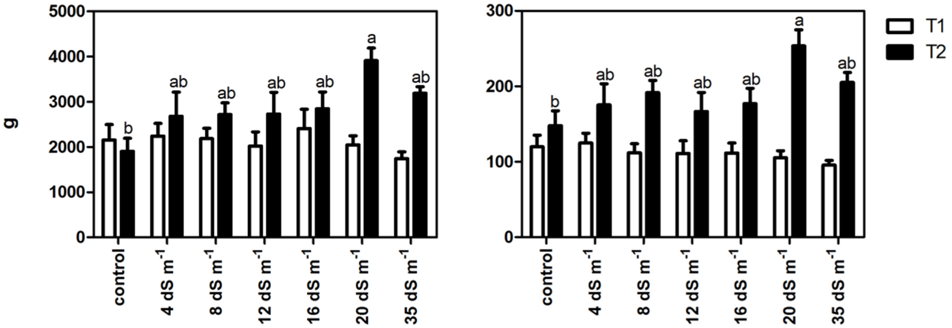


Figure 2. FW and DW of the ice plant shoot, collected at T1 and T2. Values are means ± s.e. (n = 9) expressed in grams per plant. Different letters indicate significant differences among treatments at $P < 0.01$ in FW plot and at $P < 0.05$ in DW plot (Tukey's Test).

Figure 3 reports the growth results of young fully expanded leaves only. As for total shoot biomass production (Figure 2), no significant differences in FW and DW were assessed between treatments and the control at T1. In contrast, at T2 both biomass parameters significantly increased in plants growing at saline conditions compared to the control, with increasing differences with increasing salinity: while total shoot DW had significantly increased compared to control only at the 20 dS m^{-1} treatment, a significant increase of young fully expanded leaves DW was assessed at 8, 12, 20 and 35 dS m^{-1}.

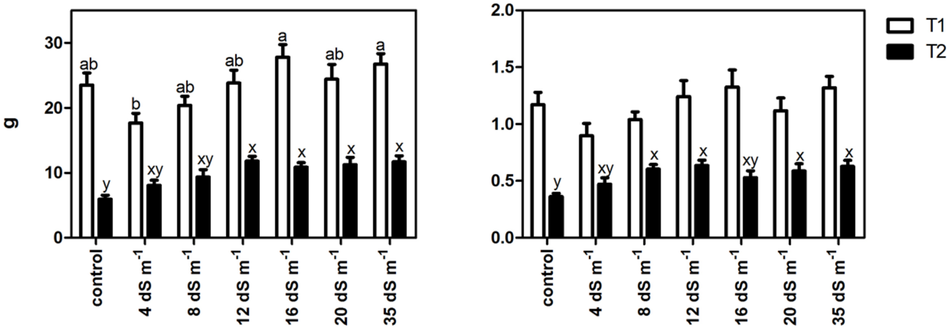
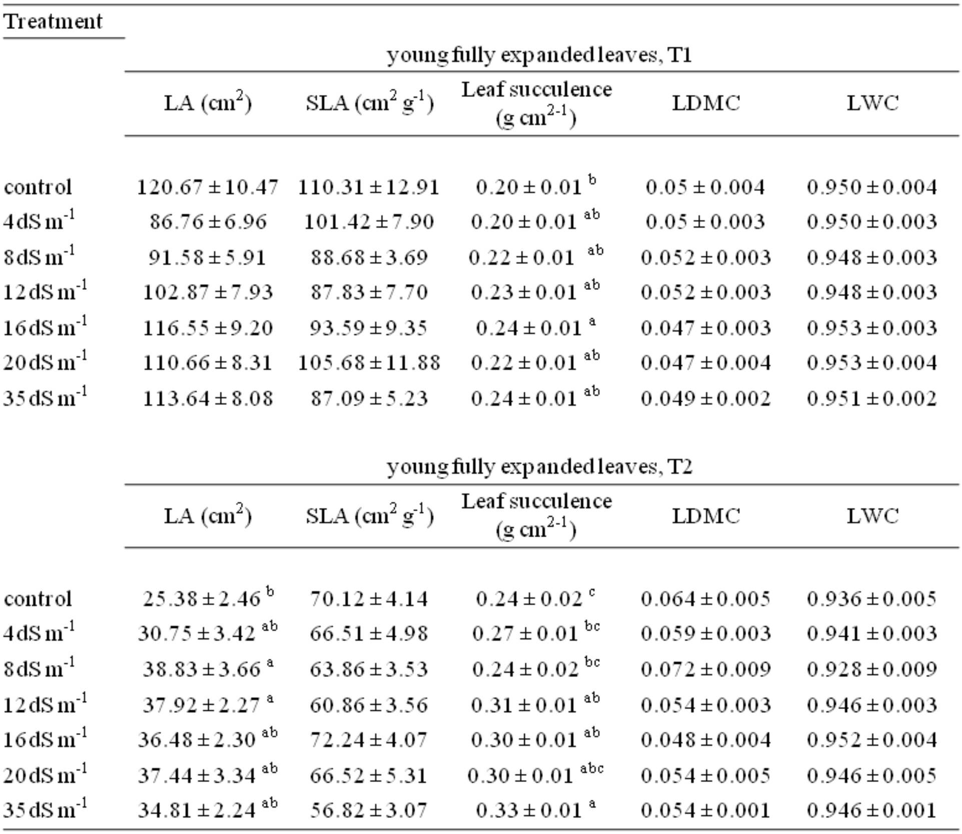


Figure 3. FW and DW of 3 young fully expanded leaves per plant, collected at T1 and T2. Values are means ± s.e. (*n* = 9) expressed in grams. Different letters indicate a significant difference among treatments at the same harvest event at $P < 0.01$ (Tukey's Test)

As shown in Table 1, increased salinity did not overall affect the morphological parameters investigated, with the only exceptions of leaf area and succulence. LA increased significantly at T2 and only at the intermediate salinity treatments compared to the control, whereas leaf succulence increased in treated plants compared to the control at T1 in 16 dS m^{-1} treatment plants and at T2 starting from 12 dS m^{-1} treatment up to the maximum salinity level, with the exception of the 20 dS m^{-1} treatment. On the other hand, SLA, LDMC and LWC did not show any significant difference among treatments at any sampling event.

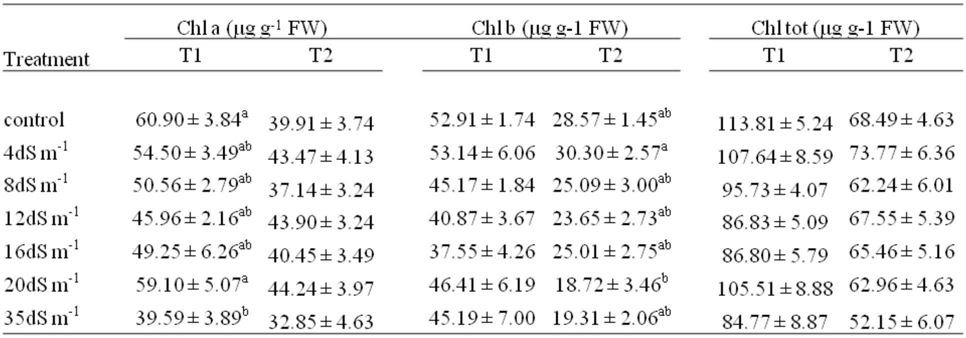
Table 1: LA, SLA, leaf succulence, LDMC and LWC of 3 young fully expanded leaves per plant, collected at T1 and T2. Values are means ± s.e. ($n = 9$). Different letters in the same column indicate a significant difference at $P < 0.05$ (Tukey's Test).



Treatment	young fully expanded leaves, T1				
	LA (cm^2)	SLA (cm^2 g^{-1})	Leaf succulence (g cm$^{2-1}$)	LDMC	LWC
control	120.67 ± 10.47	110.31 ± 12.91	0.20 ± 0.01 ^{b}	0.05 ± 0.004	0.950 ± 0.004
4 dS m^{-1}	86.76 ± 6.96	101.42 ± 7.90	0.20 ± 0.01 ^{ab}	0.05 ± 0.003	0.950 ± 0.003
8 dS m^{-1}	91.58 ± 5.91	88.68 ± 3.69	0.22 ± 0.01 ^{ab}	0.052 ± 0.003	0.948 ± 0.003
12 dS m^{-1}	102.87 ± 7.93	87.83 ± 7.70	0.23 ± 0.01 ^{ab}	0.052 ± 0.003	0.948 ± 0.003
16 dS m^{-1}	116.55 ± 9.20	93.59 ± 9.35	0.24 ± 0.01 ^{a}	0.047 ± 0.003	0.953 ± 0.003
20 dS m^{-1}	110.66 ± 8.31	105.68 ± 11.88	0.22 ± 0.01 ^{ab}	0.047 ± 0.004	0.953 ± 0.004
35 dS m^{-1}	113.64 ± 8.08	87.09 ± 5.23	0.24 ± 0.01 ^{ab}	0.049 ± 0.002	0.951 ± 0.002
	young fully expanded leaves, T2				
	LA (cm^2)	SLA (cm^2 g^{-1})	Leaf succulence (g cm$^{2-1}$)	LDMC	LWC
control	25.38 ± 2.46 ^{b}	70.12 ± 4.14	0.24 ± 0.02 ^{c}	0.064 ± 0.005	0.936 ± 0.005
4 dS m^{-1}	30.75 ± 3.42 ^{ab}	66.51 ± 4.98	0.27 ± 0.01 ^{bc}	0.059 ± 0.003	0.941 ± 0.003
8 dS m^{-1}	38.83 ± 3.66 ^{a}	63.86 ± 3.53	0.24 ± 0.02 ^{bc}	0.072 ± 0.009	0.928 ± 0.009
12 dS m^{-1}	37.92 ± 2.27 ^{a}	60.86 ± 3.56	0.31 ± 0.01 ^{ab}	0.054 ± 0.003	0.946 ± 0.003
16 dS m^{-1}	36.48 ± 2.30 ^{ab}	72.24 ± 4.07	0.30 ± 0.01 ^{ab}	0.048 ± 0.004	0.952 ± 0.004
20 dS m^{-1}	37.44 ± 3.34 ^{ab}	66.52 ± 5.31	0.30 ± 0.01 ^{abc}	0.054 ± 0.005	0.946 ± 0.005
35 dS m^{-1}	34.81 ± 2.24 ^{ab}	56.82 ± 3.07	0.33 ± 0.01 ^{a}	0.054 ± 0.001	0.946 ± 0.001

Chlorophyll and carotenoid concentrations. In Table 2, chlorophyll a, b and total chlorophyll concentrations in young fully expanded leaves are reported for T1 and T2. Salinity generally did not affect pigments concentration, only chlorophyll a was significantly reduced in T1 35 dS m^{-1} treatment plants compared to the control. Chlorophyll decreased in time both in salt treated plants as in controls, thus not necessarily depending on salinity.

Table 2. Chlorophyll a, chlorophyll b and total chlorophyll concentration of young fully expanded leaves at T1 and T2. Values are means ± s.e. ($n = 9$) expressed in microgram per gram of fresh weight. Different letters in the same column indicate a significant difference at $P < 0.05$ (Tukey's Test).



	Chl a (μg g^{-1} FW)		Chl b (μg g-1 FW)		Chl tot (μg g-1 FW)	
Treatment	T1	T2	T1	T2	T1	T2
control	60.90 ± 3.84^{a}	39.91 ± 3.74	52.91 ± 1.74	28.57 ± 1.45^{ab}	113.81 ± 5.24	68.49 ± 4.63
4dS m^{-1}	54.50 ± 3.49^{ab}	43.47 ± 4.13	53.14 ± 6.06	30.30 ± 2.57^{a}	107.64 ± 8.59	73.77 ± 6.36
8dS m^{-1}	50.56 ± 2.79^{ab}	37.14 ± 3.24	45.17 ± 1.84	25.09 ± 3.00^{ab}	95.73 ± 4.07	62.24 ± 6.01
12dS m^{-1}	45.96 ± 2.16^{ab}	43.90 ± 3.24	40.87 ± 3.67	23.65 ± 2.73^{ab}	86.83 ± 5.09	67.55 ± 5.39
16dS m^{-1}	49.25 ± 6.26^{ab}	40.45 ± 3.49	37.55 ± 4.26	25.01 ± 2.75^{ab}	86.80 ± 5.79	65.46 ± 5.16
20dS m^{-1}	59.10 ± 5.07^{a}	44.24 ± 3.97	46.41 ± 6.19	18.72 ± 3.46^{b}	105.51 ± 8.88	62.96 ± 4.63
35dS m^{-1}	39.59 ± 3.89^{b}	32.85 ± 4.63	45.19 ± 7.00	19.31 ± 2.06^{ab}	84.77 ± 8.87	52.15 ± 6.07

On the other hand, carotenoids concentration, reported in Figure 4, augmented in time at increased salinity, whereas the control remained stable. Plants with the higher concentration of carotenoids at both harvests were those of the 20 dS m^{-1} treatment.

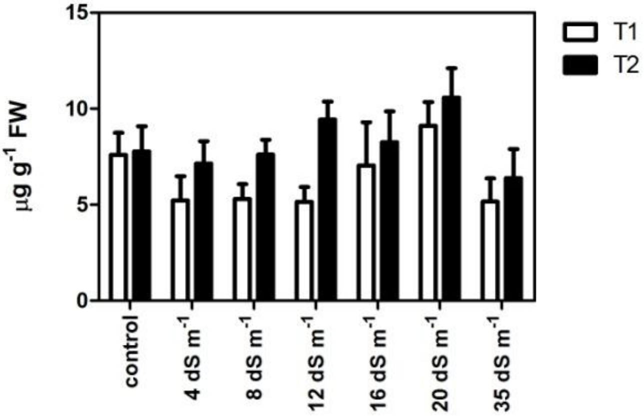
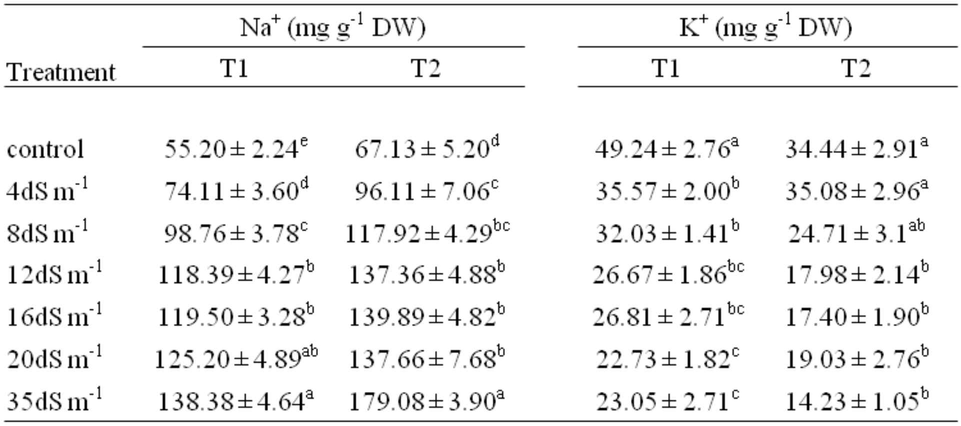


Figure 4. Carotenoid concentration of young fully expanded leaves at T1 and T2. Values are means ± s.e. ($n = 9$) expressed in microgram per gram of fresh weight. No significant differences were assessed among treatments at $P < 0.05$ (Tukey's Test).

Concentration of Na^+, K^+, and Ca^{2+}. Values of Na^+, K^+, and Ca^{2+} concentration in leaves of juvenile plants collected before treatments introduction (T0) resulted to be: Na^+ 32.82 ± 2.33; K^+ 30.11 ± 4.57; Ca^{2+} 3.16 ± 0.16 (values are means ± s.e., expressed in milligram per gram of dry weight). Table 3 shows Na^+ and K^+

concentrations in young fully expanded leaves collected at T1 and T2. Sodium concentrations were significantly higher in every treatment compared to the control, at both sampling events. On the other hand, K^+ concentration significantly declined accordingly with increasing salinity compared to the control, starting from 4 dS m^{-1} treatment at T1 and from 12 dS m^{-1} treatment at T2.

Table 3: Concentration of *Na^+ and K^+* in young fully expanded leaves at T1 and T2. Values are means ± s.e. (*n* = 9) expressed in milligram per gram of dry weight. Different letters in the same column indicate a significant difference at *P* < 0.0001 (Tukey's Test).



	Na^+ (mg g^{-1} DW)		K^+ (mg g^{-1} DW)	
Treatment	T1	T2	T1	T2
control	55.20 ± 2.24^{e}	67.13 ± 5.20^{d}	49.24 ± 2.76^{a}	34.44 ± 2.91^{a}
4dS m^{-1}	74.11 ± 3.60^{d}	96.11 ± 7.06^{c}	35.57 ± 2.00^{b}	35.08 ± 2.96^{a}
8dS m^{-1}	98.76 ± 3.78^{c}	117.92 ± 4.29^{bc}	32.03 ± 1.41^{b}	24.71 ± 3.1^{ab}
12dS m^{-1}	118.39 ± 4.27^{b}	137.36 ± 4.88^{b}	26.67 ± 1.86^{bc}	17.98 ± 2.14^{b}
16dS m^{-1}	119.50 ± 3.28^{b}	139.89 ± 4.82^{b}	26.81 ± 2.71^{bc}	17.40 ± 1.90^{b}
20dS m^{-1}	125.20 ± 4.89^{ab}	137.66 ± 7.68^{b}	22.73 ± 1.82^{c}	19.03 ± 2.76^{b}
35dS m^{-1}	138.38 ± 4.64^{a}	179.08 ± 3.90^{a}	23.05 ± 2.71^{c}	14.23 ± 1.05^{b}

Calcium concentration, reported in Figure 5, rose in treated leaves compared to the control with increasing salinity at both sampling events.

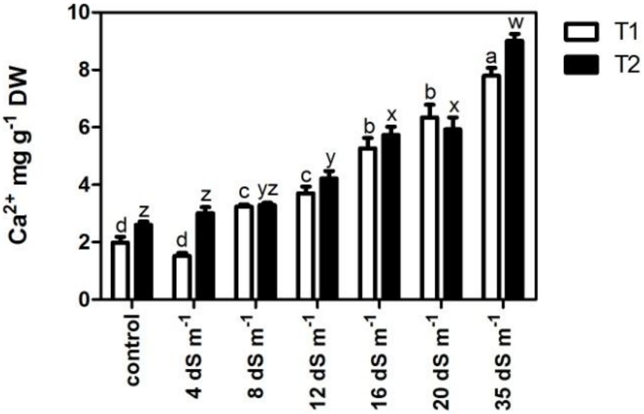
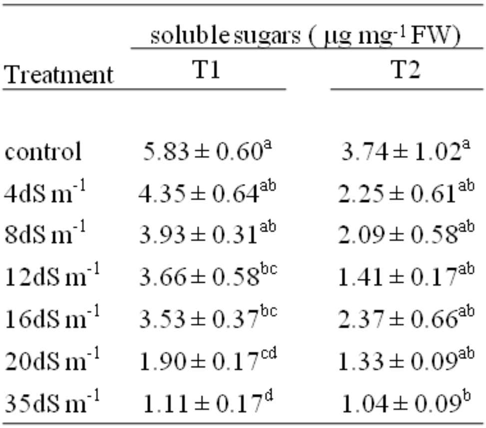


Figure 5. Concentration of Ca^{2+} in young fully expanded leaves at T1 and T2. Values are means ± s.e. ($n = 9$) expressed in milligram per gram of dry weight. Different letters indicate a significant difference among treatments at the same harvest event at $P < 0.0001$ (Tukey's Test)

Soluble sugars concentration. The soluble sugars concentration in young fully expanded leaves at T1 and T2 is reported in Table 4. At both harvests, increased salinity coincided with a decrease of soluble sugars concentrations compared to the control. At T1, the reduction was significant starting from the 12 dS m^{-1} treatment up to the higher salinity treatments. However, at T2 only at the 35 dS m^{-1} treatment a significant decrease was found. Soluble sugar concentrations proved to be more stable over time in the higher salinity treatments, while at the control and at the low to moderate salinity treatments (i.e. up to the 12 dS m^{-1} treatment), concentrations dropped from T1 to T2.

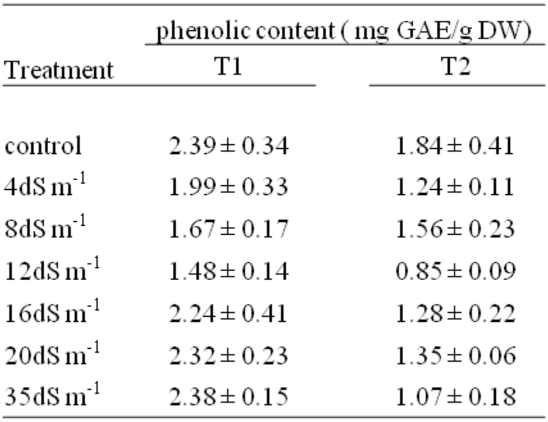
Table 4: Concentration of soluble sugars in young fully expanded leaves at T1 and T2. Values are means ± s.e. (*n* = 9) expressed in microgram per milligram of fresh weight. Different letters in the same column indicate a significant difference at *P* < 0.0001 for T1 and at *P* < 0.05 for T2 (Tukey's Test).



Treatment	soluble sugars (μg mg^{-1} FW) T1	T2
control	5.83 ± 0.60^{a}	3.74 ± 1.02^{a}
4dS m^{-1}	4.35 ± 0.64^{ab}	2.25 ± 0.61^{ab}
8dS m^{-1}	3.93 ± 0.31^{ab}	2.09 ± 0.58^{ab}
12dS m^{-1}	3.66 ± 0.58^{bc}	1.41 ± 0.17^{ab}
16dS m^{-1}	3.53 ± 0.37^{bc}	2.37 ± 0.66^{ab}
20dS m^{-1}	1.90 ± 0.17^{cd}	1.33 ± 0.09^{ab}
35dS m^{-1}	1.11 ± 0.17^{d}	1.04 ± 0.09^{b}

<u>Phenolics content and antioxidant activity</u>. The concentrations of phenolics in young fully expanded leaves at T1 and T2 are reported in Table 5. No significant differences were found among treatments at either sampling event.

Table 5: Concentration of phenolic compounds in young fully expanded leaves at T1 and T2. Values are means ± s.e. (*n* = 3) expressed in milligram of gallic acid on gram of dry weight. No letters indicate no significant differences among treatments at *P* < 0.05 (Tukey's Test).



Treatment	phenolic content (mg GAE/g DW) T1	T2
control	2.39 ± 0.34	1.84 ± 0.41
4dS m^{-1}	1.99 ± 0.33	1.24 ± 0.11
8dS m^{-1}	1.67 ± 0.17	1.56 ± 0.23
12dS m^{-1}	1.48 ± 0.14	0.85 ± 0.09
16dS m^{-1}	2.24 ± 0.41	1.28 ± 0.22
20dS m^{-1}	2.32 ± 0.23	1.35 ± 0.06
35dS m^{-1}	2.38 ± 0.15	1.07 ± 0.18

Figure 6 shows the volumes of extracts to be added to give a 50% reduction of the stable free radical DPPH: lower values indicate higher antioxidant activity. Our results show a significant reduction of the antioxidant activity only in 12 dS m^{-1} treatment leaves compared to the control, at both sampling events. In contrast, both 20 and 35 dS m^{-1} treated leaves showed an antioxidant activity comparable to the control.

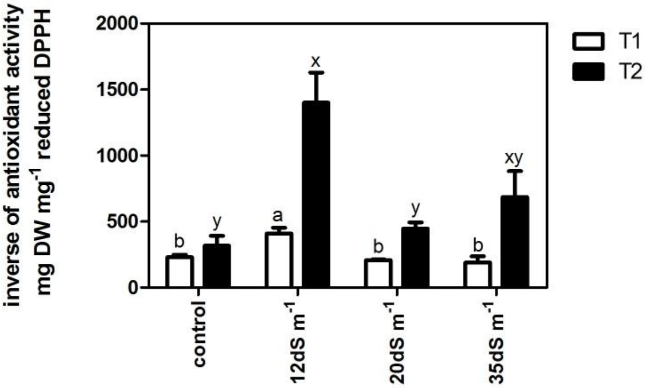


Figure 6. Inverse of the antioxidant activity of young fully expanded leaves at T1 and T2. The lower value indicates the higher antioxidant activity . Values are means ± s.e. (*n* = 3) and are expressed in mg of dry weight needed to reduce of the 50% 1 mg of the stable free radical DPPH. Different letters indicate a significant difference among treatments at the same harvest event at $P < 0.05$ (Tukey's Test).

4. Discussion

Seawater irrigation did not damage the ice plant growth up to EC 35 dS m^{-1}. Our experimental results indicated that none of the six treatments negatively affected the growth of the ice plant in terms of biomass production as compared to the control. On the contrary, the better performances of plant biomass at higher salinity levels may suggest a sort of growth stimulation by increased salinity. In fact, significant differences in total shoot biomass production were observed only at T2, with 20 dS m^{-1} salinity treatment plants showing a significant augmentation of both fresh and dry total shoot biomass compared to the control. Likewise, juvenile control leaves FW and DW were lower than salt-treated ones: FW and DW almost doubled in higher salinity treatments compared to control. This is an important result, given that in saline agriculture settings, the young leaves are the ones to be harvested.

In this sense, *Mesambryanthemum crystallinum* does not deviate from other halophytes that have their best performances in the presence of NaCl. In fact, for a number of dicotyledonous halophytes optimal growth occurs at concentrations of 50-250 mM NaCl (roughly corresponding to EC 5 and 25 dS m^{-1}) in the root medium (Flowers and Colmer 2008). Moreover, halophytes generally do not occur on non-saline sites because of their reduced growth rate and reduced competitive ability at low salinity conditions (Rozema and Schat 2013). Our growth results are in accord with Herppich and co-workers (2008), who did not find significant biomass differences in *Mesambryanthemum* plants grown at 150 mM NaCl, roughly comparable to EC 15 dS m^{-1}, and those grown with tap water for the first 105 days of the experiment, while a significantly higher fresh biomass in salt-treated plants compared to control was found at day 150. The good performance of the ice plant throughout the higher salinity levels indicate its potential for saline agriculture.

High salinity extended the ice plant growing season. A second important impact of saline conditions on the ice plant is that its growing season was strongly extended under seawater irrigation. Control plants started to senesce in August, to proceed to the seed production phase. In contrast, the same senescence started in high salinity treatments plants about one month after, being in August still on the vegetative growth phase. The reason of the control smaller biomass compared to high salt treated plants may thus partly be found in the differences in vegetative cycle length. Control plants ended their life cycle before the salt treated ones, thus at T2 controls were already starting to shrink, unlike the salt treated plants. Likewise, Adams et al. (1998) reported that salinity slow down the plant developmental physiology. This is particularly important for our experiment purpose of testing the ice plant as potential salt tolerant crop in saline agriculture. In the particular case of the ice plant, the edible part is represented by young leaves, thus the ice plant harvest is not a destructive one, as only young leaves are picked leaving the plant undamaged. Concerning total shoot and juvenile leaves change in biomass from T1 to T2, differences are found with total shoot biomass increasing and juvenile biomass on the contrary decreasing from T1 to T2. The increase in total shoot biomass can be explained by the presence at T2 of old leaves with considerable area extension and of long shoots grown during the whole plant cycle. On the other hand, the ideal moment of harvest of juvenile leaves was identified in July because at that time leaves had the higher biomass at both saline and non saline conditions. However, as salinity proved to extend the whole vegetative stage, this also enables one extra month of harvest compared to non salinity conditions. In addition, the number of young leaves produced, thus the grams of potential harvest, were increased by the extended cycle length, as the ice plant is a fast growing species: in fact, even if smaller in T2 compared to T1, juvenile leaves were harvestable and thus marketable at August sampling too.

Growth and morphological response to increased salinity. Over time, the morphological changes were expressed more strongly with leaf succulence increasing with increasing salinity. This capability may represent an essential part of the ice plant salt tolerance. In fact, halophytes balance their growth rate with their requirement for the salt needed for osmotic adjustments (Flowers and Yeo 1986). The increase in leaf succulence, thus of the water content per unit area, is part of this balance and plays a major role in the osmotic adjustment to a low external water potential induced by salinity (Flowers and Colmer 2008). Also, it translates to an augmented carbon assimilation capacity per unit area, assuring plants growth despite a possibly relatively low SLA (de Vos et al., 2013). Indeed, in dicotyledonous halophytes the increase in leaf succulence is often connected to a SLA decrease, (Rozema et al. 2015; de Vos et al. 2013; de Vos et al. 2010; Geissler, Hussin, and Koyro 2009; Ayala and O’Leary 1995), a morphological adaptation associated with the plants need of limiting transpiration (Flowers and

Flowers 2005). Nevertheless, as reported in the results section, no significant decrease of SLA occurred in treated plants compared to the control, nor in LDMC and LWC. This is in line with Herppich, Huyskens-Keil, and Schreiner (2008), and confirms that none of the treatments did effectively stress the plant, but increased its physiological activity and yield. In fact, in our experiment LA of salt-treated leaves rose compared to the control (even if significantly only at the intermediate salinity treatments). It can be suggested that the ice plant leaf area was not reduced by salinity because another feature helped in regulating the leaf ion concentration: the bladder cells, filled with a water solution and functioning as peripheral salinity and water reservoirs (Agarie et al., 2007; Luttge et al., 1978). It is clear that in the agricultural field settings used here, the morphological parameters related to salt tolerance responses were induced with increasing salinity. Leaf succulence and the glistening bladder cells, in particular, provides the edible leaves with a taste, consistence and appearance that make it particularly appreciated by consumers (authors personal observations).

Physiological and osmotic adaptations to salinity. The shoot color differences among treatments observable in Figure 1 might be related to the differences in chlorophyll concentration. Indeed, both chlorophyll a and b decreased, even if overall not significantly, in the higher salinity treatment 35 dS m^{-1} and in the intermediate salinity ones. The impacts are, however, not strong, because these changes did not negatively affect the plants' photosynthetic apparatus, nor therefore the plants' growth. Our field experimental results are in line with previous lab studies reporting a decrease in chlorophyll pigments in various halophytes species at saline conditions (Aghaleh et al., 2009; Ayala and O'Leary, 1995; Parida et al., 2002). Focusing on the ice plant, Barker et al. (2004) found overall no significant differences between high and low salt treated plants.

The concentrations of Na^+, K^+ and Ca^{2+} found in control juvenile plants (T0) was in line with data reported in literature (Adams et al., 1998; Agarie et al., 2007).

Results on Na^+ and Ca^{2+} concentration in salt treated plants proved to be in accord with literature as well: Agarie et al. (2007) reported a comparable sodium concentration in *M. crystallinum* leaves treated with 0 and 400 mM NaCl (roughly corresponding to EC of 40 dS m^{-1}, slightly above our higher salinity treatment). The sodium concentrations coincide with the strategy of *Mesembryanthemum* being a sodium includer, with an increasing sodium gradient from roots to shoot apices (Bohnert and Cushman 2000). Interestingly, adult leaves accumulate more Na^+ than young ones: even if juvenile leaves increase their salt content and the associated inorganic and organic ions under salinity conditions, all ions are accumulated to a lower concentration compared to adult leaves stressed for the same period (Adams et al., 1998). At maturity the ice plant accumulates and compartmentalizes sodium in its bladder cells, which sequester salt from the photosynthetic tissues that are contributing to the seed formation (Adams et al., 1998), leaving them unaffected by salinity.

Within agricultural settings, the strategy to accumulate sodium in predominantly old instead of young leaves may also be profitable because this aspect might prevent the edible leaf product from having a too high sodium content, that could otherwise have negative effects for human health. The accumulation of sodium comes at the cost of accumulating K^+ and resulted in Na^+/K^+ ratios of around six in our experiment (again coinciding with literature: Agarie et al. 2007; Ghnaya et al. 2005; Adams et al. 1998; Demmig and Winter 1986; Harvey et al. 1981). However, it has also been shown that a cytosol Na^+/K^+ ratio smaller than 1 does not seem generally essential for halophyte tolerance to high salinity conditions (Demmig and Winter, 1986). Also the increased calcium concentrations with increasing salinity (Agarie et al. 2007; Adams et al. 1998; Yang et al. 2007) coincide with the salt tolerance of the ice plant as experimental evidence correlated Ca^{2+} increase with salt adaptation (Parida and Das 2005): calcium is believed to protect membranes structure and function under salt stress (Yan et al., 1995), and its concentration increase under salinity stress can ameliorate the inhibitory effect on growth (Epstein, 1972). Moreover, the increased

Ca^{2+} concentration seems to act as a second messenger that results in changes in gene expression and metabolism in salt-affected cells (Sairam and Tyagi 2004).

Besides the accumulation or exclusion of cations, the salt tolerance of halophytes is often associated with several osmotic adjustments that lead to the accumulation of a number of organic solutes, as for example soluble sugars (Parida et al., 2002; Wang et al., 2013). Nevertheless, variations in soluble carbohydrates presence under saline conditions are not well understood and information on physiological events involved in this process is scarce (Prado et al., 2000). In fact, free sugar responses to such stress are conflicting, both in glycophytes and in halophytes (Gorham et al., 1981). Our experimental results did not seem to indicate soluble sugars as solutes produced by the ice plant to cope with salinity. In fact, soluble sugars decreased, at both harvests, with increasing salinity (Keiller et al., 1987). According to Briens and Larher (1982), halophytes do not necessarily produce high concentrations of carbohydrates as a response to salinity, and several solutes that would be able to act as osmoregulatory metabolites exist, i.e. proline and polyols (myo-inositol, pinitol, ononitol), that also have importance for nutrition (Livesey, 2003; Wu et al., 2011).

Furthermore, the absence of significant differences in phenolics concentration between salt-treated plants and the control proves once again that none of the tested salt concentrations was stressful for the ice plant. Salinity thus did not lead to an increase on phenolics nor of the antioxidant activity of the ice plants extracts. This can be explained by *M. crystallinum* capability of avoiding the C_3 mode of carbon fixation by switching to CAM. In general, this seems to be one of the main strategies utilized by halophytes to decrease ROS production while maintaining photosynthesis during stress (Bose et al., 2013). Also thanks to the efficient mechanisms of Na^+ exclusion from the cytosol, salt-tolerant species may not require a high level of antioxidants because they do not allow excessive production of ROS (Bose et al., 2013).

Nutritive quality of ice plant with increased salinity. The significant increase of Ca^{2+} concentration may represent an interesting nutritional improvement achievable in salinity conditions. Calcium is among the main mineral elements lacking in the diet of over two-thirds of the world's population (White and Broadley 2009). To address this issue, agronomic approaches optimizing mineral fertilization to increase the concentrations of several mineral elements in agricultural products are of interest (Lynch, 2007). The ice plant is able to acquire and accumulate important mineral elements and the saline environment provided the appropriate conditions to enrich its calcium concentration. Encouragingly, a strong correlation exists between Ca^{2+} and Mg^{2+} plants accumulation capability (White and Broadley 2009). Furthermore, species from families within the Caryophyllales tend to accumulate uncommonly high Mg^{2+} leaf concentrations (White and Broadley 2009; Broadley et al. 2004; White 2001), as well as showing remarkable shoot Zn^{2+} concentration, which is generally higher in Caryophyllales in comparison with other plants orders (White and Broadley 2009). Accordingly, investigations on possible patterns between salinity and other mineral elements with an important role in human diet (i.e. Cu^{2+}, Fe^{2+}, Mg^{2+}, Zn^{2+}) could add important information on the ice plant nutritional enrichment opportunity in a saline environment.

Furthermore, the carotenoids - another nutritive goal - rose between T1 and T2 in all salt treated plants, while no increase was found in the control. At each harvest, the carotenoids concentrations were unaffected by saline conditions (this study, Barker et al. 2004). Also in this feature, the ice plant seems to distinguish itself positively compared to some other halophyte plants in which the carotenoid concentration may decrease with increasing salinity (Aghaleh et al., 2009; Qiu et al., 2003; Redondo-Gomez et al., 2010).

Perspective of ice plant crop in saline agriculture. As none of the tested salt concentrations has resulted in biomass loss, it seems possible to cultivate *M. crystallinum* in saline agriculture, up to a salinity level characterized by EC of 20-35 dS m^{-1}. Perhaps even higher salinity levels are possible since we did not identify a threshold of biomass reduction, although the highest biomass production was suggested to occur around an EC of 20 dS m^{-1}. The already appreciated taste of saline agriculture vegetables in different countries (Rozema and Schat 2013), and of the ice plant in particular, helped by its glistening appearance (Agarie et al., 2009; Herppich et al., 2008), also encourage such possibility. At the higher salinity levels of 20-35 dS $m^{-1}$the vegetative cycle of the species proved to be extended, enabling a longer productive phase, thus higher yield. According to our results, only the Na^+ concentration in the edible leaves could constitute a concern for the healthy characterization of the crop: monitoring its content would prevent possible health issues.

5. Conclusions

This species' ability to achieve remarkable growth rates under saline conditions validates its crop potential in saline environments. The results of this study provide a clear evidence that the production of the ice plant with edible purposes can be obtained in saline conditions characterized by EC of 20 - 35 dS m^{-1} with an extended growing season and high nutritive characteristics. Also, the increased leaf succulence and glistening bladder cells at increased salinity provide the edible leaves with a taste, consistence and appearance particularly appreciated by consumers.

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Chapter 5. General discussion and conclusions

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Seawater as a complementary or alternative water source

Agriculture should be at the center of global efforts of adaptation to climate change because it is essential for our food supply and it depends directly on natural resources, which are inextricably linked to climate (FAO, 2016). Furthermore, the current demographic growth rate will lead to a world population of up to 10.9 billion people by the end of the century (UN, 2013), with the consequent need to increase food production of about 70% (FAO, 2016, 2009). Yet, agriculture is a high-demanding resources sector, in particular concerning freshwater, with 70% to 90% of total available freshwater absorbed by this sector (FAO, 2011, 1988; SDSN, 2013). Important differences in the amount of water needed for the production of different foods do exist, i.e. about 1830 liters of water are needed to produce a kilogram of wheat (Mekonnen and Hoekstra, 2011) whereas approximately 15400 for a kilogram of beef (Mekonnen and Hoekstra, 2012). Nevertheless, to ensure the food security goal, particular attention will be needed in every food production system, not only in the more resources demanding. In this perspective, new sustainable agricultural systems are necessary to adapt to current or expected climate changes, minimizing their negative effects and possibly taking advantage of the opportunities created (FAO, 2016).

In this thesis, the possibility of using seawater for irrigation was explored. Seawater is in fact the most abundant source of water of the planet and its specific composition represents a very well balanced ionic environment for plants (Boyko, 1966). In fact, with the exception of its very high chloride content (about 75% NaCl and 10% of $MgCl_2$), seawater is rich in all nutritive elements needed by plants, including the necessary trace elements and micro-organisms, living or dead (Boyko and Boyko, 1966; Eyster, 1968). The described experimental results assessed such possibility as a concrete option to obtain a competitive production by replacing a part of fresh water with sea water for the investigated species, and encouragingly for many others. Nevertheless, particular attention is deserved by the environmental factors. Accordingly, in this thesis, seawater is tested and suggested only in soilless systems or on soils naturally affected by seawater infiltration, as for example coastal areas. Following those attentions to preserve the soil resource, seawater introduction as complementary or alternative water source can allow food production while saving freshwater and, in the meanwhile, exploiting seawater as a nutrient supply for plants growth.

Seawater and glycophytes

In Chapter 2 the amounts of seawater not compromising the growth and production of three important crops were determined. Lettuce (*Lactuca sativa* L. var. Canasta), Swiss chard (*Beta vulgaris* L.) and chicory (*Cichorium intybus* L.) showed remarkable differences in their tolerance to seawater salinity and thus in their productivity. In particular, lettuce growth was negatively affected starting from 10% seawater irrigation, whereas for chard and chicory none of the tested salinity levels (up to 15% seawater irrigation) resulted in any loss of productivity compared to control conditions. To explain those differences, the chard and chicory moderate salt tolerance has been possibly retained from their salt resistant ancestors (Shannon and Grieve, 1998). Such salinity thresholds were also confirmed by physiological parameters as the photosynthetic rate and the chlorophyll concentration in leaves, supporting that the administered quantities of seawater did not represent stressful conditions for the plants growth. Interestingly, water consumptions dropped and WUE significantly upturned in every tested crop accordingly with increased seawater concentrations. Also, salinity led to an interesting enhancement in the accumulation of mineral elements. For example, chard was the crop with the higher number of elements (Na^+, Ca^{2+}, Cu^{2+} and Mg^{2+}) with increased concentration in treated leaves compared to the control. 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 nutritional improvement achievable in saline conditions. All results overall suggest that the use of a certain level of seawater in the cultivation of lettuce, chard and chicory is a practical possibility in the direction of increasing both crop WUE and the concentration of some mineral nutrients.

The water saving goal achieved irrigating with seawater emerged in Chapter 3 too, by means of the comparison of the water footprint of chicory grown in different cropping systems: open field cultivation, conventional NFT hydroponic and seawater NFT hydroponic, i.e. with a share of 10% seawater in the growing media. Significant differences were observed between each soilless system compared to open field cultivation, assessing for hydroponics-grown chicory a reduction of 63.9% and for sea-hydroponics a reduction of 74.5%. Interestingly, significant differences were assessed between the two soilless systems too, with the sea-hydroponics achieving a water footprint reduction of 29.5% compared to conventional hydroponics. Importantly, such best performance in water saving did not come at a cost of biomass production, which was not negatively affected. Accordingly, seawater in the growing media enhanced the water use efficiency of the tested species, and implies, for certain crops, the nutritive improvement of the edible product, as for example the enhanced accumulation of mineral elements reported in Chapter 2.

Seawater and halophytes

Halophytes can withstand remarkably higher amounts of seawater compared to glycophytes. Results of the field experiment reported in Chapter 4 proved that seawater irrigation did not damage *Mesambryanthemum crystallinum* growth up to EC of 20-35 dS m^{-1}, or perhaps even higher, since a threshold of biomass reduction was not identified. On the contrary, the better performances of plant biomass at higher salinity levels may suggest a sort of growth stimulation by increased salinity. In this sense, the ice plant does not deviate from other halophytes that have their best performances in the presence of NaCl. A second important impact of saline conditions on the ice plant is that its growing season was strongly extended under seawater irrigation: while control plants started to senesce in August, to proceed to the seed production phase, the same senescence started in high salinity treatments plants about one month after. This is particularly important for the experiment purpose of testing the ice plant as potential salt tolerant crop in saline agriculture. Being the edible part represented by young leaves, the ice plant harvest is not a destructive one, as only young leaves are picked leaving the plant undamaged. Accordingly, as salinity proved to extend the whole vegetative stage, this also enables one extra month of harvest compared to non salinity conditions. In addition, the number of young leaves produced, thus the grams of potential harvest, were increased by the extended cycle length, as the ice plant is a fast growing species. Together with quantitative improvement, also qualitative ones were obtained. For example, leaf succulence increasing with increasing salinity, together with the glistening bladder cells on leaves, filled with a water solution and functioning as peripheral salinity and water reservoirs (Agarie et al., 2007; Luttge et al., 1978), provide the edible leaves with a taste, consistence and appearance that make it particularly appreciated by consumers. Moreover, the significant increase of Ca^{2+} concentration in edible leaves may represent an interesting nutritional improvement achievable in salinity conditions. Calcium is in fact among the main mineral elements lacking in the diet of over two-thirds of the world's population (White and Broadley 2009). Encouragingly, a strong correlation exists between Ca^{2+} and Mg^{2+} plants accumulation capability (White and Broadley 2009). Accordingly, investigations on possible patterns between salinity and other mineral elements with an important role in human diet (i.e. Cu^{2+}, Fe^{2+}, Mg^{2+}, Zn^{2+}) could add important information on the ice plant nutritional enrichment opportunity in a saline environment. Similarly, the carotenoids - another nutritive target - rose between the two plants samplings in all salt treated plants, while no increase was found in the control. This species’ ability to achieve remarkable growth rates under saline conditions validates its crop potential in saline environments. Coastal areas, in particular, are among the most vulnerable zones to climate change consequences. Yet, they seem ecologically optimal to host the cultivation of halophytes as new crops in a saline agriculture context.

Conclusions and perspectives

The results of this thesis provide a clear evidence on the possibility of growing crops by using seawater as a complementary or alternative water source. Of course, different amounts of seawater have to be considered according to the chosen crop. This consideration implies that different species and varieties need to be tested to understand their particular salinity threshold, as no a priori screening seems adequate. Our results on three glycophytes already proved remarkable differences among different species, but also suggested the possibility to use up to 15% of seawater share in the irrigation supply for some of them. For halophytes, on the other hand, the amounts of seawater usable would be remarkably more consistent, opening to the possibility of designate lands naturally affected by salinity, i.e. coastal areas affected by seawater infiltration, to the production of new crops in a saline agriculture context. Seawater share would be responsible for a decrease in freshwater demand, an increase of crops water use efficiency, and the enhancement of mineral elements and secondary metabolites that may lead to a nutritional value improvement of products.

In conclusion, despite glycophytes and halophytes differences in salt tolerance, the use of seawater can be suggested as a controlled complementary irrigation source. This scenario offers several advantages, as an increased sustainable food production achievable exploiting seawater or brackish waters and salt-affected lands, resources that would not compete with traditional agriculture and that seems to be destined to grow as a consequence of climate change. Furthermore, because of the strategies adopted by plants to cope with salinity, crops grown in saline conditions could also be a source of compounds with a potential added nutritive and economic value.

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Chapter 6. Summary

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Agriculture is at the center of global efforts of adaptation to climate change because of two main reasons: it is essential for our food supply and it depends directly on natural resources, which are inextricably linked to climate. Available freshwater represents about 1% of water on Earth, yet food production requires on average 70% of such a small freshwater share, reaching up to 90% in the Middle-East and North Africa (MENA) regions. Furthermore, a remarkable increase in food production is forecast because of the growing population, increasing even further the human pressure on the water resource. On the other hand, seawater represents 97% of total water on the planet, and its specific composition constitutes a very well balanced ionic environment for plants: with the exception of its very high chloride content, seawater is rich in all nutritive elements needed by plants, including the necessary trace elements and micro-organisms, living or dead. As production is still competitive by replacing a part of freshwater with seawater, its use in agriculture can represent an answer to limit the use of freshwater and, in the meanwhile, to exploit seawater as a nutrient supply for plants growth. New sustainable agricultural systems are in fact necessary to adapt to current or expected climate changes, minimizing their negative effects and possibly taking advantage of the opportunities created. Accordingly, saline agriculture can represent a climate change adaptation strategy.

In Chapter 2, the possibility of growing lettuce (*Lactuca sativa* L. var. Canasta), Swiss chard (*Beta vulgaris* L.) and chicory (*Cichorium intybus* L.) with seawater and freshwater blends in hydroponic conditions was explored. In particular, the crops were grown using 5%, 10% and 15% of seawater, corresponding to the following electrical conductivity (EC) values: 3 dS m^{-1}, 6 dS m^{-1}, 9 dS m^{-1}. Crops growth, water consumptions and water use efficiency (WUE) were investigated to assess the maximum salinity tolerance thresholds of the different species. Photosynthetic activity and stomatal conductance were evaluated too on a weekly basis to monitor the plants responses to the seawater irrigation. At the end of the trial, principal mineral elements, soluble sugars, carotenoids and phenolics concentration in the edible leaves were determined at the different salinity levels. While lettuce growth was negatively affected starting from 10% seawater irrigation, for chard and chicory none of the tested salinity levels resulted in any loss of productivity compared to control conditions. Such salinity thresholds were also confirmed by the physiological parameters and the chlorophyll concentration in leaves, supporting the fact that the administered quantities of seawater did not represent stressful conditions for the plants growth. Interestingly, water consumptions dropped and WUE significantly upturned in every tested crop accordingly with increased seawater concentrations. Also, salinity led to an interesting enhancement in the accumulation of mineral elements. For example, chard was the crop with the higher number of elements (Na^+, Ca^{2+}, Cu^{2+} and Mg^{2+}) with increased concentration in treated leaves compared to the control. 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 nutritional improvement achievable in saline conditions. All results overall suggest that the use of a certain level of seawater in the cultivation of lettuce, chard and chicory is a practical possibility in the direction of increasing both crop WUE and the concentration of some mineral nutrients.

In Chapter 3, different cropping systems for food production were compared on the basis of their need in water by using the water footprint (WF) indicator, that indicates the overall amount of freshwater involved in the production cycle of any good or service. In particular, chicory was the case study crop chosen and its need in water during the whole production was compared among open field cultivation, conventional hydroponics and seawater hydroponics, using a share of 10% of seawater in its growing media. The water saving goal emerged in Chapter 2 is confirmed in this Chapter as well. In fact, significant differences were observed firstly between each soilless system compared to open field cultivation, assessing for hydroponics-grown chicory a reduction of 63.9% and for sea-hydroponics a reduction of 74.5%. Interestingly, significant differences were assessed between the two soilless systems too, with the sea-hydroponics achieving a water footprint reduction of 29.5% compared to conventional hydroponics. Importantly, such best performance in water saving did not come at a cost of biomass production, which was not negatively affected. Accordingly, seawater in the growing media remarkably enhanced the water use efficiency of the tested species.

In Chapter 4, the effects of increasing levels of seawater salinity irrigation were assessed on the growth and productive performance of the salt-tolerant edible halophyte *Mesembryanthemum crystallinum*, the common ice plant, under field conditions. Six treatments were used in the experiment, each one repeated in three plots, characterized by the following electrical conductivities (EC) values: 4dS m^{-1}, 8dS m^{-1}, 12dS m^{-1}, 16dS m^{-1}, 20dS m^{-1}, and 35dS m^{-1}. An additional control treatment (repeated in three plots as well) was characterized by EC of 2dS m^{-1}. Together with the ice plant crop potential, a full screening of morphological and physiological characteristics was assessed to investigate eventual adaptations to salinity. The effects of different salinity levels on 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 were determined too, i.e. mineral elements, carotenoids, soluble sugars, phenolic compounds and the antioxidant activity. Seawater irrigation did not damage *Mesambryanthemum crystallinum* growth up to EC of 20-35 dS m^{-1}, or perhaps even higher, since a threshold of biomass reduction was not identified, whereas the better performances of plant biomass at higher salinity levels may suggest a sort of growth stimulation by increased salinity. Furthermore, the ice plant growing season was extended of about one month under seawater irrigation. This is particularly important for the experiment purpose of testing the ice plant as potential salt tolerant crop in saline agriculture: as salinity proved to extend the whole vegetative stage, this also enables one extra month of harvest compared to non salinity conditions. Together with quantitative improvement, also qualitative ones were obtained. For example, leaf succulence increasing with increasing salinity together with the glistening bladder cells on leaves provide the edible leaves with a taste, consistence and appearance that make it particularly appreciated by consumers. Moreover, the significant increase of Ca^{2+} concentration in edible leaves may represent an interesting nutritional improvement achievable in salinity conditions. Similarly, the carotenoids rose between the two plants samplings in all salt treated plants, while no increase was found in the control. This species' ability to achieve remarkable growth rates under high levels of seawater irrigation validates its crop potential in saline environments. Coastal areas, in particular, are among the most vulnerable zones to climate change consequences. Yet, they seem ecologically optimal to host the cultivation of halophytes as new crops in a saline agriculture context.

In Chapter 5, the general discussions and the conclusions of the preceding chapters are synthesized and discussed together. The chapter is concluded by stressing the importance for the agriculture sector to adapt to current or expected climate changes. Accordingly, saline agriculture is suggested as a complementary source for sustainable food production achievable exploiting seawater or brackish waters and salt-affected lands, resources that would not compete with traditional agriculture and that seems to be destined to grow as a consequence of climate change.

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