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Effect of seawater concentration on the productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia* halophytes as leafy vegetable crops

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ABSTRACT

The halophyte *Salicornia* was recently introduced as a fresh vegetable crop that thrives in extreme salt conditions. Two annual *Salicornia* and two perennial *Sarcocornia* ecotypes were investigated for yield production and nutritional value in response to different seawater concentrations in the irrigation solution. A harvest schedule based on a three-week cycle gave better productivity than a two-week or a four-week cycle. Total yield declined with increasing percentage of seawater above 50% in the irrigation water, however annual plants had always ca 2–3-fold higher fresh biomass in comparison to their perennial counterparts. Increased percentages of seawater in the irrigation solution had the following effects on ion concentrations in the shoots: no change in Ca^{2+} and Mg^{2+}, a slight increase in K^+, and marked elevations in Na^+ and Cl^-. Importantly, total polyphenol, β-carotene and ureides, all known for their antioxidant capacities, rose with increasing seawater percentage, findings that indicated improved nutritional values for *Salicornia* and *Sarcocornia* irrigated with high concentrations of seawater. Impressively, both the annual *Salicornia* and the perennial *Sarcocornia* ecotypes exhibited high total shoot lipid contents of up to 2.41 and 2.06 mg g^{-1} fresh weight, respectively, which included an omega-3 fraction of 47.6 and 41.2% of the total fatty acid content. Moreover, the high fatty acid content of the annual *Salicornia* ecotype was not significantly affected by increasing seawater concentrations. In this study, we thus demonstrated the feasibility of cultivating *Salicornia* and *Sarcocornia* by applying a multiple harvest system and 100% percentages of seawater in the irrigation water generating economic yields with high nutritional value. The findings also showed that *Salicornia* and *Sarcocornia* leafy vegetables may attract additional interest as an alternative source of omega-3 polyunsaturated fatty acids for human consumption, even when the crop irrigated solely with seawater.

1. Introduction

The limited resources of fresh water for agriculture and the ongoing increase in soil salinity throughout the world demands the development of new crops that are able to tolerate higher salt concentrations than conventional agricultural crops (Yensen, 2006; Glenn et al., 1999). In tandem, there is a necessity to develop new agro-techniques for the application of highly saline irrigation adapted to novel halophyte crop plants (Glenn et al., 1998). Promising candidates for the development of novel halophytes as crop species are *Salicornia* and *Sarcocornia*, genera that thrive naturally along coastal salt marshes from the Arctic to the Mediterranean and that are often subjected to daily tides. Species of both genera, which express extreme salt tolerance, are often referred to as pioneer plants on the sea coasts (Davy et al., 2001, 2006). *Salicornia* plants are characterized by a simple morphology since they produce only succulent shoots that are apparently leafless. The genus *Sarcocornia* is now distinguished from the annual *Salicornia* by its distinct perennial growth habit (Davy et al., 2006) and differences in the flower arrangement (Kadereit et al., 2007).

Salicornia has been introduced into the European market as a vegetable with leafless shoots resembling green asparagus. The young fleshy tips of this green vegetable are in high demand in gourmet kitchens, not only for their salty taste, but also for their high nutritional value in terms of minerals and antioxidant vitamins, such as vitamin C and β-carotene (Lu et al., 2001). Nevertheless, there is very little information available about cultivation

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conditions and their effect on the nutritional value and the product quality of *Salicornia* ecotypes. Furthermore, at present the available plant material comprises a collection of ecotypes of different origins; there are no selected 'varieties' with desirable properties.

Salt stress is the major environmental factor limiting plant growth and productivity (Parida and Das, 2005). However, halophyte plants have developed an array of mechanisms to accommodate the stress, including the compartmentation of Na^+ in the vacuole, which is mediated by the active driving force of Na^+/H^+ antiporters (Parks et al., 2002; Glenn et al., 1999). To maintain the ionic balance in the vacuoles, the synthesis of osmotically active metabolites is enhanced. Hence, sugars, such as fructose, glucose and sucrose, accumulate in the leaves of halophytes in response to salt stress (Parida et al., 2002). The increased production of these organic compounds and the active transport of toxic ions across the vacuolar membrane constitute a considerable energetic cost, which results in the reduction of both growth and biomass production (Greenway and Munns, 1980).

Salt stress also leads to the increased formation of reactive oxygen species, which may disrupt inter alia the integrity of cellular membranes and the activities of various enzymes (Zhu, 2001). In response to this type of oxidative damage, halophytes induce the synthesis of antioxidant enzymes and non-enzymatic antioxidant compounds, e.g. polyphenols, ascorbic acid, β-carotene and ureides (Brychkova et al., 2008; Parida and Das, 2005). Enhanced tolerance to salt stress can further be achieved by an increase in the unsaturation of the fatty acids in the chloroplast membrane, the major portion of which comprises α-linolenic acid (18:3ω3) in many plants (Allakhverdiev et al., 1999; Simopoulos, 2004). The combination of antioxidant compounds with fatty acids having a high content of omega-3 fatty acids thus contributes to the nutritional value of crop plants.

With the exception of a study on the perennial shrub *Atriplex triangularis* (Gallagher, 1985) many years ago, very little work has been conducted on multiple-harvest regimes for fresh vegetable production from halophyte plants. In this study, we investigated the cultivation of annual *Salicornia* and perennial *Sarcocornia* ecotypes in a multiple harvest system. We investigated the effects of increasing the seawater content in the growth medium and were able to show economically viable yields of plants with a high nutritional value under irrigation with full-strength seawater.

2. Materials and methods

2.1. Plant material and growth conditions

Two *Salicornia persica* and two *Sarcocornia fructicosa* ecotypes were investigated. The two annual *Salicornia* types DS and RN (cultivated commercially in Israel) originated in the Dead Sea area of Israel (Ventura et al., 2010). The two *Sarcocornia* types were also collected in Israel, EL on the northern Mediterranean coastline and VM in the Ramat HaNegev district.

Experiments implementing seawater-based irrigation were carried out in a temperature-controlled greenhouse in Beer Sheva, Israel. Summer temperatures were kept below 33 °C, via a cooling system, while in the winter the greenhouse was heated when temperatures dropped below 20 °C. The photoperiod was fixed to a 15-h day-length by using 100-W standard light bulbs to prevent flowering under short-day light conditions.

Seawater was prepared by dissolving 33 g of Red Sea Salt® (Red Sea Fish Pharm Ltd., Eilat, Israel; www.redseafish.com) in 1 L water, according to the manufacturer's instructions. Different relative seawater (RSW) treatments (25, 50, 75% seawater) were obtained by diluting the complete seawater solution with tap water (EC 0.7 mS m^{-1}). The irrigation solutions for all the seawater concentrations were supplemented with 200 ppm commercial N–P–K fertilizer (20–20–20 + microelements, Haifa Chemicals Ltd., Israel). Tap water supplemented with the same fertilizer concentration served as the control. The pH values ranged between 7.3 and 7.5 for all seawater combinations. Seeds were sown directly on perlite (Agrekal Habonim Industries Ltd., Moshav Habonim, Israel; www.agrekal.co.il), and after emergence, the seedlings were exposed gradually – over one month – to the final seawater levels.

2.1.1. Experimental set up

Salt concentrations of 0, 25, 50, 75, and 100% seawater were applied in a series of experiments. In a pot experiment, five 0.5-L plastic pots containing perlite were placed in 18-L boxes (dimensions 37 cm × 31 cm × 16 cm), each of which was then filled with the same amount of seawater nutrient solution of 0, 25, 50, 75, and 100% concentration. The solutions were changed every second day. A single pot contained 20–30 plants, and each treatment was replicated five times. In a subsequent group of experiments, plants were grown hydroponically in 50-L polystyrene cultivation boxes (dimensions 82 cm × 37 cm × 18 cm) containing perlite. Three 50-L boxes were fitted into one hydroponic unit (dimension 118 cm × 94 cm × 17 cm). Each unit was filled with the same amount of 50, 75 or 100% seawater nutrient solution, into which air was pumped with an aquarium pump. For the duration of the experiment, the solutions were topped up at the ends of odd weeks (starting at week 1). At the ends of even weeks, the solutions were completely replaced. Each salt treatment was performed in two replicas, giving a total of 18 50-L boxes. The four plant ecotypes were replicated three times randomly within the salinity treatments. A uniform plant density of 1000 seedlings per m^2 was obtained.

2.2. Germination experiment

Seeds were placed in 9-cm Petri dishes lined with a double layer of filter paper (Whatman No.1) and incubated in 0, 25, 50, 75 or 100% seawater (same volumes) at 25 °C with a 12-h photoperiod. Germination was evaluated over six days, and germinated seedlings were counted every second day. Seeds were considered as having germinated when a 1-mm radicle had emerged from the seed. Each treatment was performed in three replications.

2.3. Growth parameters

Five to eight single plants were collected four months after sowing. Plant material was separated into shoots and roots. Fresh weight, plant height and root length were determined.

2.4. Harvest regime

To compare different harvest regimes, 20–30 plants per pot were irrigated with 100% seawater nutrient solution, as described above. Plants were cropped at time intervals of two, three and four weeks over a period of five months, giving 10, 6 and 5 total harvests, respectively. By two months after sowing, the plants had reached a marketable size of about 15 cm height, and were cut approximately 5 cm above the surface, thereby producing a cutting table. Subsequent harvests were carried out by cutting the plants just above the cutting table. After determination of fresh weight, the shoots were oven dried at 70 °C to constant weight. Total yield per square meter was calculated in terms of the fresh weight accumulated by each single harvest per unit area. Total dry biomass was determined as the percentage of dry weight of a known fresh sample.

2.5. Cation and anion determination

Dry shoot samples were digested in concentrated HNO_3 and H_2O_2 (10:1). Leaf digests were filtered through Millex®-GP 0.22 μm filter units prior to cation analysis, which was performed by ICP–OES (inductively coupled plasma–optical emission spectrometry, Perkin–Elmer Optima 3000, PerkinElmer Inc., Waltham, MA).

Anions were extracted in water with the addition of 200 ppm ascorbic acid. After extraction, samples were heated at 95 °C for 2 min to eliminate organic matter and subsequently centrifuged at 15,000 × g for 5 min. The collected supernatant was filtered through 0.2 μm Target® nylon filter units and diluted further prior to injection into an ion chromatography system (LC-20, Dionex Corporation, Sunnyvale, CA, www.dionex.com). Sample aliquots of 25 μL were separated on an IonPac® column (AS 9-SC; Dionex) and eluted with a solution containing 2 mM Na_2CO_3/0.75 mM $NaHCO_3$ at a flow rate of 2 mL min^{-1}. Anions were detected by an electrochemical conductivity detector (ED 50; Dionex) combined with an upstream-inserted anion self-regenerating suppressor (ASRS® 300 4 mm; Dionex) AutoSuppression® recycle mode.

2.6. Fatty acid profile

To determine the fatty acid profile and content, shoots were dipped for 30 s in chloroform to remove cuticular wax prior to lyophilization. The dried plant material was then transmethylated with 2% sulfuric acid in dry methanol at 80 °C over 1.5 h under an argon atmosphere. Heptadecanoic acid (C_{17}) (Sigma Chemical Co. St. Louis, MO) was added as internal standard. Fatty acid methyl esters (FAME) were then extracted with *n*-hexane, and their profiles were identified on a Thermo Ultra gas chromatograph equipped with autosampler, PTV injector, FID detector and a Zebron GC column (ZB-WAXplus 30 mL × 0.32 mm ID × 0.25 μm df), using a temperature gradient from 130 °C (hold time 1 min) to 200 °C (linear increase of 10 °C min^{-1}; hold time 7 min) and finally to 230 °C (linear increase of 15 °C min^{-1}; hold time 5–10 min) by co-chromatography with authentic standards (Sigma Chemical Co.). Helium was used as the carrier gas.

2.7. Chemical constituents

Shoot samples were snap-frozen in liquid nitrogen immediately after collection and stored at −80 °C until extraction. Shoot extraction was carried out as described by Ventura and Mendlinger (1999). Briefly, frozen leaf samples were homogenized on ice in a Polytrone (Kinematica, Switzerland) at a ratio of 1:4 (w/v) with doubly distilled water. The homogenate was centrifuged at 17,500 g for 20 min at 4° C, filtered through Whatman filter paper No. 1 and kept on ice. The electrical conductivity in the supernatant (EC [dS/m]) was measured with a conductivity meter (CyberSan 500 Con, Eutech Instruments, Singapore), and total soluble solids (TSS [%]) were determined with a digital refractometer (Atago PR-1, Atago Co. Ltd., Japan).

Total soluble proteins were extracted and estimated as previously described (Ventura et al., 2010) using the Bio-Rad Protein assay, a modification of the Bradford procedure (1976); crystalline bovine serum albumin was used as the reference compound.

Chlorophyll and β-carotene extractions were performed by incubating a leaf sample in 80% acetone (1:10, w/v) for 48 h at 4 °C in the dark. After further 1:4 (v/v) dilution of the extract, chlorophyll and β-carotene were determined with a spectrophotometer (JASCO, V-530, JASCO Inc., Mary's Court, Easton, MD) at 652 nm and 480 nm, respectively (Arnon, 1949; Ben-Amotz et al., 1988).

Polyphenols were extracted from shoot material according to Singleton and Rossi (1965) in 0.4 M phosphate buffer (pH 7) at a ratio of 1:3.75 (w/v). The extract was centrifuged at 22,500 g for 20 min at 4 °C. Aliquots of the collected supernatant were incubated with Folin-Cioclateur's phenol (Sigma Chemical Co.) and 7.5% Na_2CO_3 for 2 h at 30 °C in a water bath. Absorbance was determined spectrophoto-metrically (JASCO, V-530) at a wavelength of 765 nm. Polyphenol contents are expressed as mg gallic acid equivalents (GAE) g^{-1} fresh weight.

Ureide extraction was performed in 80% ethanol at a ratio of 1:4 (w/v), and the separate assays for allantoin and allantoic acid were performed as described by Vogels and van der Drift (1970). The resulting color formation was estimated spectrophotometrically (JASCO, V-530) at 535 nm against the respective standards. Total ureides were calculated as the sum of allantoin and allantoic acid.

2.8. Statistical analysis

Statistical analysis was performed by appropriate single or multi-factorial ANOVA (analysis of variance) using the JMP In 5.0.1a software package (SAS Institute Inc., Campus Drive, Cary, NC). Subsequently, means comparisons were performed according Tukey–Kramer HSD (honestly significant difference) or Student's *t*-test with the same software.

3. Results

3.1. Germination rate

Germination was reduced as seawater concentration was increased and reached values of <20% of the control germination rate at full-strength seawater (Fig. 1). At seawater concentrations of ≥50%, ecotypes differed significantly in their germination rates: EL and RN showed the highest rates of germination in RSW of 75%, while DS showed an extreme decline, resulting in only 25% germination at a half-strength seawater concentration. No germination occurred in full-strength seawater for this ecotype, while the other ecotypes did not significantly differ in their germination rates, which ranged between 5 and 15% (Fig. 1).

3.2. Growth parameters

The effect of different seawater concentrations (0, 25, 50, 75 and 100%) on single-plant performance was determined in pot experiments for two ecotypes, RN and EL, as representative ecotypes of the annual *Salicornia* and perennial *Sarcocornia*, respectively. Completely omitting seawater from the culture medium resulted in significantly shorter plants with lower biomass accumulation in all plants. The absence of seawater also resulted in the lowest shoot-to-root ratio for both types (Table 1). For *Salicornia* RN, maximum shoot fresh weight was obtained at 75% seawater, while no significant effect of seawater concentration was found for *Sarcocornia* EL, as long as the nutrient solution contained at least 25% of seawater. For both ecotypes, seawater concentration had no effect on root weight or length. However, the shoot-to-root weight ratio increased gradually with increasing salinity in *Salicornia*, whereas no clear pattern was found for *Sarcocornia.*

3.3. Harvest regime

Since harvesting of the *Salicornia* shoots results in re-growth of new young shoot tips after cutting, we applied different harvest regimes to determine the optimal harvest intervals for maximum yields at the highest salt concentration. *Salicornia* and *Sarcocornia* ecotypes RN and EL, as representatives of ecotypes with annual and perennial growth characteristics respectively, were cropped at a two-, three- or four-week harvest cycle. The harvest regime had a significant effect on the biomass production of *Salicornia* RN,

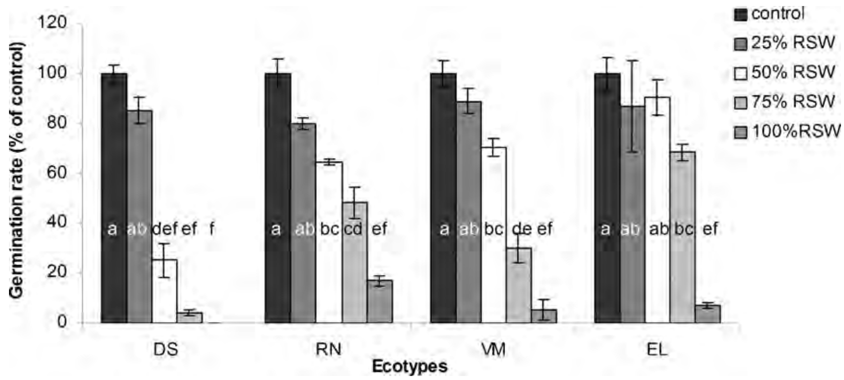

Fig. 1. Effect of different relative seawater (RSW) concentrations on the germination rate of two *Salicornia* (DS, RN) and two *Sarcocornia* ecotypes (VM, EL). Values are means ± SE ($n = 3$). Statistical analysis was performed by two-factorial ANOVA. Values followed by different letters are significantly different according to Tukey HSD, $p < 0.05$.

Table 1
Shoot and root fresh weight (FW), plant height, root length and shoot:root ratio of *Salicornia* (RN) and *Sarcocornia* (EL) ecotypes cultivated in pots supplied with different relative seawater (RSW) concentrations. Plants were sampled four months after sowing. Values are means ($n = 5–8$). Values followed by different letters are significantly different, $p < 0.05$.

Ecotype	RSW (%)	Shoot		Root		Shoot:root ratio
		FW (g plant^{-1})	Height (cm)	FW (g plant^{-1})	Length (cm)	
RN	0	1.27c	18.16b	0.076	10.46	15.6c
	25	2.86b	24.25a	0.077	11.46	36.6b
	50	3.42ab	22.32a	0.077	9.82	44.9a
	75	3.90a	23.72a	0.099	14.12	45.4a
	100	2.90b	21.00ab	0.056	13.02	48.5a
EL	0	0.15b	5.90b	0.017	5.72	18.9b
	25	0.83a	13.30a	0.016	5.03	53.3a
	50	0.74a	12.50a	0.014	7.53	56.1a
	75	0.76a	10.60a	0.016	8.10	49.5a
	100	0.70a	11.00a	0.016	5.80	45.8a

which accumulated the highest yield in the three-week regime (Fig. 2). Cutting the plants more frequently resulted in a clear decline in both the accumulated yield and in the yield per single harvest (Fig. 2). Conversely, the harvest regime had no effect on total biomass in the perennial *Sarcocornia* EL, possibly as a result of the overall lower yield obtained for this ecotype, which was about 3-fold lower than that for the annual *Salicornia* RN.

3.4. Biomass accumulation

To simulate potential commercial cultivation units, two ecotypes of *Salicornia* and *Sarcocornia* were evaluated in a hydroponic system supplied with different concentrations of seawater, ranging from half- to full-strength seawater. Increasing the seawater content resulted in a reduction in accumulated yield, which was more apparent in the annual types DS and RN, especially when cultivated on full-strength seawater (Fig. 3A). The perennial ecotypes VM and EL exhibited significantly lower total yields than the annual types, but the salinity level of the irrigation solution had no effect on yield accumulation.

Increasing the seawater content in the growth medium did not have a significant effect on dry biomass accumulation, although the ecotypes clearly differed in their dry matter accumulation: the highest dry biomass accumulation was observed in the annual

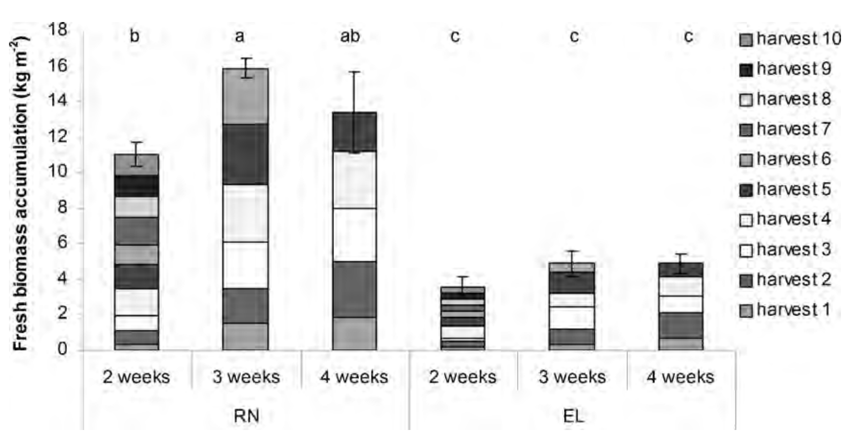

Fig. 2. Fresh biomass accumulation in *Salicornia* (RN) and *Sarcocornia* (EL) for three harvesting regimes (every 2, 3 or 4 weeks). Plants were cultivated in a hydroponic system supplied with complete seawater for six months. Values are means ± SE ($n = 4$). Statistical analysis was performed by two-factorial ANOVA. Values followed by different letters are significantly different according to Student's t-test, $p < 0.05$.

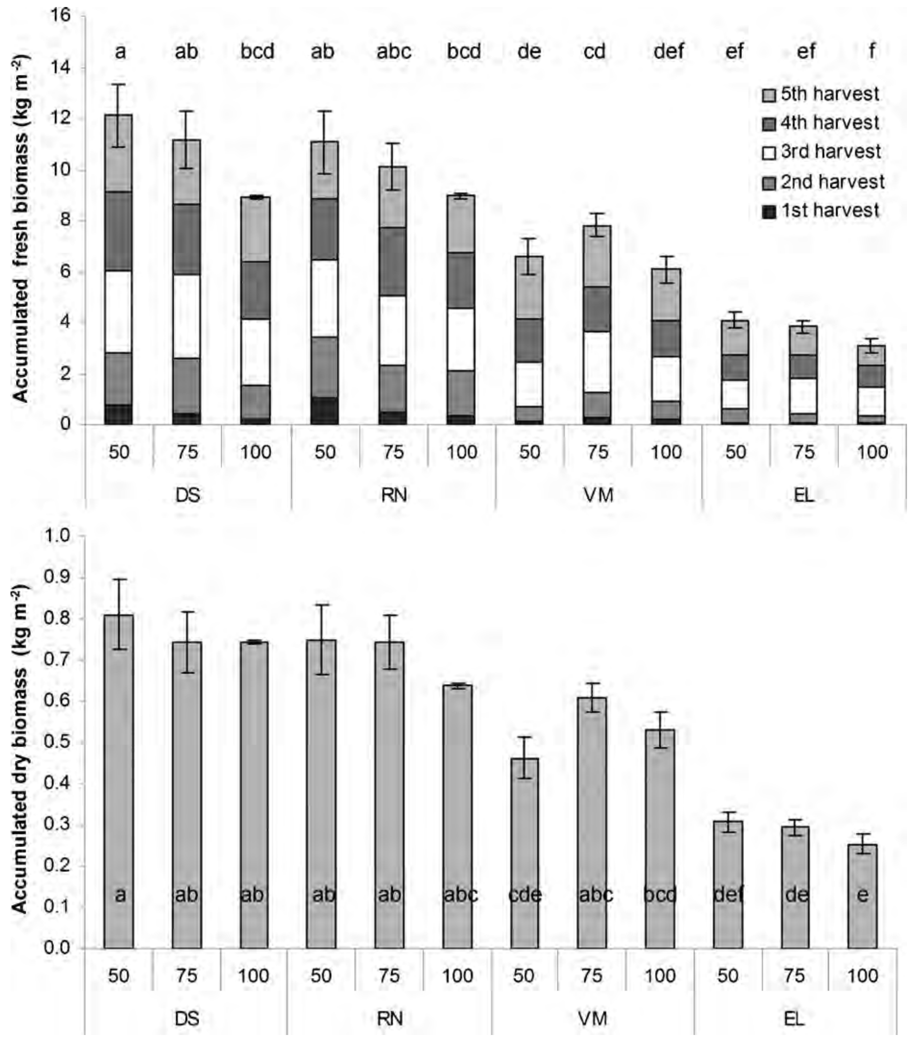

Fig. 3. Fresh (A) and dry (B) biomass accumulation of two *Salicornia* (DS, RN) and two *Sarcocornia* ecotypes, (VM, EL) cultivated in hydroponic system supplied with three relative seawater concentrations (50, 75, 100% RSW) during a six-month growing period. Values represent means ± SE ($n = 3$). Statistical analysis was performed by two-factorial ANOVA. Values followed by different letters are significantly different according to Tukey HSD, $p < 0.05$.

Salicornia, with *Sarcocornia* VM being intermediate, and EL accumulating the lowest amount of biomass (Fig. 3B).

3.5. Cation and anion content in Salicornia and Sarcocornia shoots

A comparison of the response of *Salicornia* (RN) and *Sarcocornia* (VM) to increasing seawater concentrations showed a marked buildup of Na^+ and Cl^- in *Salicornia* and *Sarcocornia* shoots. Concomitantly, K^+, Mg^{2+} and Ca^{2+} increased in the annual RN, while PO_4^- decreased significantly in the perennial VM (Table 2). There were no major changes in the contents of NO_3^- and SO_4^{2-} in response to salinity. Overall, differences in ion content were more pronounced between the *Salicornia* and *Sarcocornia* than between the salinities, with ecotype VM accumulating higher amounts of most of the measured ions (Table 2).

3.6. Shoot fatty acid content

For fresh shoot tissues, a comparison of lipid contents (based on TFA determination) and FAME profiles of *Salicornia* RN and *Sarcocornia* VM showed the highest fatty acid (FA) content in RN irrigated with 100% seawater—2.41 mg g^{-1} FW. This value was higher (but not significantly so) than the highest value of 2.06 mg g^{-1} FW obtained for VM irrigated with half-strength seawater (Table 3). The most abundant FA was α-linolenic acid (18:3ω3), which ranged from 41.2 to 48.2% of the total FA content for VM and RN, respectively, followed by linoleic acid (18:2) and palmitic acid (16:0). Additional minor FAs were also identified. Increasing the seawater concentration had no effect on the lipid content and FA composition of *Salicornia* RN, but in *Sarcocornia* VM the total and omega-3 fatty acid contents decreased significantly as the salinity was increased (Table 3).

3.7. Chemical quality

The chlorophyll content of ecotypes DS and VM was not affected by increasing seawater concentrations (Fig. 4). For the *Salicornia* ecotype RN, the maximum chlorophyll content was found at the lowest salt concentration, with a subsequent dose-dependent decline, indicating the sensitivity of this ecotype to a high salt concentration. In contrast, the perennial *Sarcocornia* EL showed a significant enhancement in chlorophyll content with increasing seawater concentration in the growth medium (Fig. 4).

Electrical conductivity (EC), a parameter indicative of mineral accumulation and saltiness, and total soluble solids (TSS), which

Table 2
Cation and anion content in the shoots of *Salicornia* (RN) and *Sarcocornia* (VM) ecotypes grown in pots supplied with increasing relative seawater concentrations (50, 75, 100% RSW). Cation and anion concentrations are expressed as mean mg g^{-1} FW ($n = 3$). Values followed by different letters are significantly different, $p < 0.05$. Upper case letters indicate the differences between salinities within ecotypes, lower case letters indicate the differences between the ecotypes.

Ecotype	RSW (%)	K^+	Na^+	$Mg_2{}^+$	$Ca_2{}^+$	$NO_3{}^-$	$PO_4{}^-$	$SO_4{}^-$	Cl^-
RN	50	2.71B	11.75Ca	0.79Cb	0.39Bb	1.57	0.80a	0.49b	22.86B
	75	2.73B	13.50Ba	0.87Bb	0.39Bb	2.45	1.05a	0.56b	24.57B
	100	3.03A	15.57Aa	1.00Ab	0.47Ab	1.89	0.75a	0.53b	29.58A
VM	50	2.58AB	11.42Ca	1.05a	0.52a	2.58	0.58ABa	0.94a	22.53B
	75	2.51B	12.56Bb	1.10a	0.61a	2.53	0.61Ab	1.07a	24.95AB
	100	2.86A	14.98Ab	1.23a	0.63a	2.27	0.44Ba	1.06a	28.46A

Table 3
Fatty acid methyl ester (FAME) profile of *Salicornia* (RN) and *Sarcocornia* (VM) grown in pots supplied with increasing relative seawater concentrations (50, 75, 100% RSW). Values in the table are mean fatty acid content (mg g^{-1} FW) ($n = 3$). Values followed by different letters are significantly different, $p < 0.05$.

RSW (%)	Ecotype RN			Ecotype VM		
	50	75	100	50	75	100
16:0	0.44ab	0.44ab	0.47a	0.42ab	0.42ab	0.40b
16:1	0.06a	0.07a	0.08a	0.05b	0.05b	0.05b
18:0	0.04	0.04	0.04	0.04	0.04	0.04
18:1ω9	0.04a	0.04a	0.05a	0.03b	0.03b	0.02b
18:2	0.55	0.51	0.55	0.56	0.56	0.48
18:3ω3	1.12a	1.07ab	1.15a	0.91bc	0.86cd	0.72d
Others	0.073	0.075	0.069	0.044	0.045	0.042
Total fatty acids	2.32a	2.24a	2.41a	2.06a	2.00a	1.76b

represents an estimate for the sweetness of the product (and accretion of compatible organic solutes), increased significantly with increasing seawater concentration in all ecotypes, but both were always higher in the *Sarcocornia* than the *Salicornia* ecotypes (Table 4).

Metabolites with antioxidant attributes constitute a major protectant for cellular structures and were therefore determined in the different *Salicornia* and *Sarcocornia* ecotypes. In general, polyphenol and ureides increased with increasing RSW for most of the ecotypes (Table 4). For β-carotene, a significant enhancement of 20% in the highest salt treatment was noticed only in *Sarcocornia* EL. Common to all ecotypes, soluble proteins showed a tendency to increase with increasing salinity, attaining maximum values at full-strength seawater. Of interest are the differences between the *Salicornia* and *Sarcocornia* ecotypes. Polyphenols and protein contents in *Sarcocornia* VM were significantly higher than those found for *Salicornia* RN. *Salicornia* ecotypes exhibited low ureide contents, which comprised only about half the values found in the *Sarcocornia* ecotypes EL and VM (Table 4).

4. Discussion and conclusions

Annual *Salicornia* species are highly salt tolerant but vary in their responses to salinity, especially during the germination process (Khan et al., 2000). High salt concentrations reduce the water potential of the medium and thus impede water uptake of imbibing seeds. Therefore, numerous halophyte seeds are sensitive to hyper-saline conditions and will germinate only after relief of the salt stress to a certain threshold (Woodell, 1985). Indeed, the germination rate gradually declined with increasing salt levels in all four *Salicornia* and *Sarcocornia* ecotypes (Fig. 1). Moreover, ecotypes differed significantly in germination rates, for salinity levels above 50% seawater concentration and ecotypes EL and RN seem to be the most tolerant. From an applied point of view, Gallagher (1985) recommended to establish a cultivation of the halophyte *Atriplex* with the irrigation of fresh water during the early germination process. We suggest that germinating seeds of *Salicornia* and *Sarcocornia* can be irrigated with seawater concentrations of up to 75%. Depending on the ecotype, reduction

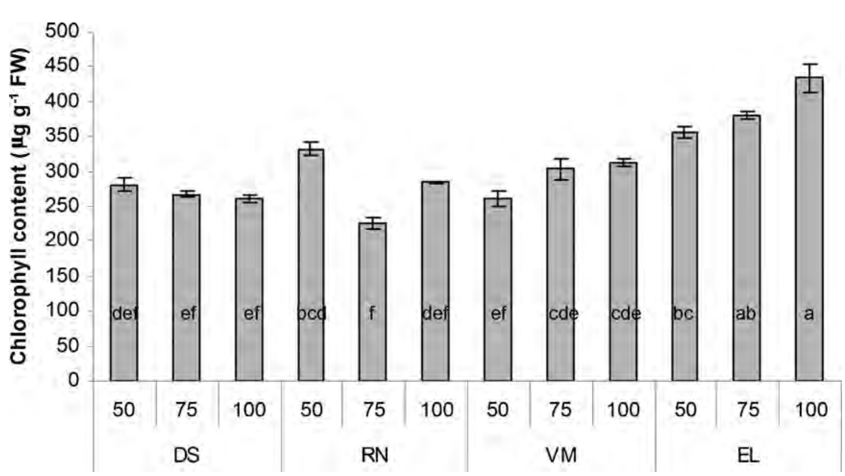

Fig. 4. Chlorophyll content of young shoot tips of two *Salicornia* (DS, RN) and two *Sarcocornia* ecotypes (VM, EL) grown in a hydroponic system supplied with three different relative seawater concentrations (50, 75, 100% RSW). Values are means ± SE ($n = 3$). Statistical analysis was performed by two-factorial ANOVA. Values followed by different letters are significantly different according to Tukey HSD, $p < 0.05$.

Table 4
Effect of relative seawater (RSW) concentration on quality parameters in two *Salicornia* (DS, RN) and two *Sarcocornia* (VM, EL) ecotypes. Values are means ($n = 3$). Values followed by different letters are significantly different, $p < 0.05$. Upper case letters indicate the differences between salinities within ecotypes, lower case letters indicate the differences between ecotypes.

Quality parameters	RSW (%)	Ecotype			
		DS	RN	VM	EL
EC (dS m^{-1})	50	27.9Bc	31.5Bb	36.5Ca	35.7Ca
	75	38.8Aab	37.0Bb	41.0Bab	42.1Ba
	100	42.9Aa	44.9Aa	48.2Aa	46.6Aa
TSS (%)	50	5.3Bb	5.1Bb	6.8Ba	6.3Ba
	75	5.7Bb	5.9ABb	6.8Ba	6.9ABa
	100	6.1Aa	6.4Aa	7.9Aa	7.2Aa
Total polyphenols (mg GAE g^{-1} FW)	50	1.37Ac	1.05Bd	1.95ABa	1.49Ab
	75	1.36Ac	1.14ABd	1.83Ba	1.57Ab
	100	1.53Ac	1.21Ad	2.05Aa	1.71Ab
β-carotene (μg g^{-1} FW)	50	46.6Ab	54.5Ab	49.9Ab	56.6Ba
	75	46.0Ab	38.8Bb	48.5Ab	60.3Ba
	100	43.9Ab	46.9ABb	50.8Ab	67.5Aa
Total ureides (ng g^{-1} FW)	50	3.35Cb	3.41Bb	7.21Ba	8.36Ba
	75	5.06Bb	5.77ABb	9.24Aa	9.73ABa
	100	6.80Ab	7.76Ab	10.17Aa	12.5Aa
Protein (mg g^{-1} FW)	50	2.93ABab	2.10Ac	3.24Ba	2.55Ab
	75	2.88Bab	2.04Ac	3.58Aa	2.80Ab
	100	3.39Aab	2.53Ac	3.59Aa	2.98Ab

in germination rate with increasing salinity should be taken into account.

A common feature of plant response to salinity is the inhibition of growth and productivity. Slower growth is an adaptive characteristic for plant survival under stress, since it allows plants to rely on multiple resources (e.g., building blocks and energy) to combat the stress (Zhu, 2001). Nevertheless, numerous halophyte species are not only able to tolerate salinity, but their growth is often stimulated by NaCl (Yousif et al., 2010). However, the optimum salt concentration for plant growth, even of the most tolerant species, is reportedly below that of full strength seawater (Flowers and Colmer, 2008). In agreement with previous findings, complete seawater caused a reduction of *Salicornia* shoot growth; however, total yields accumulated to values exceeding 8 kg m^{-2} with full-strength seawater irrigation in the annual ecotype (Fig. 3) during a six-month growing cycle, thus exceeding that of other halophyte crops (Glenn et al., 1999). This finding emphasizes the high potential of *Salicornia* as a crop species.

Since the annual *Salicornia* ecotypes gave, on average, 1.8- and 2.8-fold higher yields than the perennial *Sarcocornia* VM and EL, respectively (Fig. 3), it is anticipated that annual *Salicornia* ecotypes will exhibit faster growth rates than their perennial counterparts during short growing seasons. Whether biomass production of the perennial *Sarcocornia*, which is characterized by slow initial growth rates, would surpass *Salicornia* over extended cultivation periods is a question that remains to be addressed in longer term experiments. The significance of this question from an agricultural point of view lies in the potential to provide sustainable cultivation of a cash crop in highly saline environments all year round.

For halophytes, multiple harvest regimes are used typically for forage crops and are only occasionally applied for vegetable production (Gallagher, 1985; Glenn et al., 1999). For *Salicornia*, maximum yields were obtained with a three-week harvest regime (Fig. 2). These yields outperformed the biomass production of other salt-tolerant crops and of two freshwater-irrigated forage plants (Glenn et al., 1998). These findings therefore refute the notion that halophytes are inherently slow-growing plants. Importantly, the high yield performance highlights the advantage of using a multiple-cropping system for leafy vegetable production for the halophyte *Salicornia*.

A decline in the content of the cations K^+, Ca^{2+} and Mg^{2+} with increasing Na^+ availability has been noted for both halophyte and non-halophyte plants, while augmented Cl^- contents are believed to have antagonistic effects on NO_3^- uptake (Yousif et al., 2010). Despite growth with full-strength seawater, both *Salicornia* and *Sarcocornia* did not show a decline in cation content (Table 2), supporting the existence of a well-defined nutrient uptake system that enables massive NaCl compartmentation while keeping an efficient balance of the additional macro nutrients needed for proper and rapid plant growth.

In comparison to other leafy vegetables irrigated with moderate salt levels, such as New Zealand spinach, water spinach and *A. hortensis* (Wilson et al., 2000; Yousif et al., 2010), *Salicornia* and *Sarcocornia* have a high content of minerals, even when grown with full-strength seawater. As *Salicornia* is a new fresh vegetable for human consumption, product quality is a major concern. *Salicornia* shoots are not only a good source of minerals, but they also contain proteins and various vitamins (Lu et al., 2001). Furthermore, the nutritional value of some, but not all, ecotypes (Table 4) was enhanced by increasing seawater concentration, which resulted in higher contents of the antioxidant compounds (polyphenols, β-carotene) and proteins.

Elevated EC values in the shoots (Table 4), resulting from high seawater concentrations in the irrigation water, may contribute to the salty taste of the crop, thus improving produce quality, particularly in *Sarcocornia*, which has higher EC values than the *Salicornia*. It should be noted that EC values were significantly lower for the first harvest than for the two subsequent harvests, also indicating an improvement of the produce quality with time (data not shown).

Omega-3 fatty acids, which are a major constituent of plant lipids located in the chloroplast membrane, are known for their beneficial properties for human health (Simopoulos, 2004). Both *Salicornia* and *Sarcocornia* had higher total lipid and omega-3 contents than spinach, lettuce and mustard green leaves (Simopoulos, 2004). Nevertheless, the representative *Salicornia* ecotype excelled the *Sarcocornia* not only with its higher absolute total FA values, but also its omega-3 percentage was not affected by increasing seawater concentration (Table 3). Thus, *Salicornia* ecotypes may attract considerable interest as an alternative source of polyunsaturated FAs for human consumption, even when grown on full-strength seawater.

The assessment of chlorophyll provides a measure of the green vegetable color preferred by consumers and of the senescence induced by salt stress. Salinity may result in damage to the photosynthetic apparatus (Parida and Das, 2005), which is ultimately expressed as lower chlorophyll contents. In all ecotypes tested, except for RN, salinity had either no effect or even a positive effect on chlorophyll content, findings supporting the extreme salt tolerance of *Salicornia* and *Sarcocornia* (Fig. 4).

The biosynthesis of ureides rather than amides and their transport from the roots to the shoots under stress conditions is energetically less costly in terms of C/N ratio (Smith and Atkins, 2002). Therefore, we suggest that *Salicornia* and *Sarcocornia* take advantage of the carbon- and energy-conserving ureide transport strategy by synthesizing allantoin and allantoic acid in the roots, where carbon is limited. From a nutritional point of view, the production of ureides is advantageous, since these compounds have significant antioxidant capacity (Brychkova et al., 2008). The high ureide content, in tandem with the other antioxidant metabolites and the rich protein levels, therefore confer additional nutritional value on *Salicornia* and *Sarcocornia*, when grown as a vegetable crop with full-strength seawater (Table 4).

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