agriculture

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Growth, Phenolics, Photosynthetic Pigments, and Antioxidant Response of Two New Genotypes of Sea Asparagus (*Salicornia neei* Lag.) to Salinity under Greenhouse and Field Conditions

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Received: 17 June 2018; Accepted: 18 July 2018; Published: 23 July 2018

Abstract: Small succulent halophytic shrubs of the genera *Salicornia* and *Sarcocornia* (Salicornioideae, Amaranthaceae) are commonly named sea asparagus and consumed worldwide as green salad in gourmet food, as conserves, and beverages. Their shoots are rich in bioactive compounds and plants show high yields in a wide range of salinities, but little is known about how salt cultivation conditions affect their chemical composition. Two genotypes (BTH1 and BTH2) of the Brazilian sea asparagus *Salicornia neei* Lag. were evaluated for salt tolerance and changes in shoot concentrations of organic metabolites and antioxidant activity under different salt exposure in both greenhouse and field conditions. All greenhouse plants received full strength modified Hoagland solution in deionized water with a basic electrical conductivity (EC) of 1.7 dS m^{-1}, and with NaCl concentrations (in mM) of ~0.1 (control), 34, 86, 171, 513, and 769. After fifty days of cultivation, both *S. neei* genotypes showed high salt tolerance and grew better under low salinities (34–86 mM NaCl) than under control salinity. Shoots of BTH1 genotype appeared to be undergoing lignification and used their high carotenoid content to dissipate the oxidative power, and the zeaxanthin content and de-epoxidation state of xanthophylls (DES) were positively affected by salinity. Under increasing salinity, BTH2 genotype had higher relative content of chlorophyll b, which may have lowered the plant photo-oxidation rate, and increased shoot concentration of the flavonoid quercetin (up to 11.6 μg g^{-1} dw at 769 mM NaCl), leading to higher antioxidant capacity. In the field experiment, after 154 days of irrigation with saline (213 mM NaCl) shrimp farm effluent, BTH2 plants grew taller, produced more metabolites (e.g., total phenolics, total free flavonoids, quercetin, and protocatechuic acid) and had a greater antioxidant capacity of shoots than that of BTH1 plants and that of traditional crops irrigated with fresh water. Yield and bioactive compound composition of *S. neei* genotypes' shoots can be enhanced by cultivation under moderate saline conditions.

Keywords: halophyte; salt stress; functional food; shrimp farm effluent; breeding program

1. Introduction

Plants of the genera *Salicornia* and *Sarcocornia* (Salicornioideae, Amaranthaceae) are small succulent shrubs with leafless stems and branches commonly named sea asparagus, glasswort or

samphire [1,2]. The most recently worldwide phylogenetic treatment of Salicornioideae based on molecular markers, according to Piirainen et al. [3], proposed merging of perennial species of the genus *Sarcocornia* in three subgenera under *Salicornia*. These plants are halophytes found worldwide in salt marshes and salt deserts, are consumed as green gourmet salads, preserves, and beverages (nuruk, wine and vinegar) [2,4]. Cultivation of sea asparagus has been performed in different parts of the world (e.g., USA, Mexico, Israel, Saudi Arabia, Eritrea and Brazil) with aquaculture saline effluent, and brackish and/or seawater as both experimental and commercial crops [2,4,5]. In general, sea asparagus showed high yields in a wide range of salinities, but each species presents its own singular growth responses under increasing saline stress [4,6,7].

Besides the high content of crude protein, fatty acids (e.g., oleic, linoleic and palmitic acids) and minerals [5,8–10], sea asparagus shoots are also rich in bioactive organic metabolites, such as phenolic acids (e.g., caffeic, ferulic, and syringic), flavonoids (e.g., quercetin and kaempferol) [8,11,12], vitamin C [12] and carotenoids [10,13]. Consequently, the consumption of these plants can produce numerous health benefits to humans, such as protection against oxidative stress, stimulation of immune responses, prevention of weight gain, and accumulation of lipids [2,14]. Production of organic functional compounds by sea asparagus, as by other halophytes, results mainly from their physiological adaptations to survive under salt stress and its associated effects (e.g., ionic, osmotic and oxidative stress) [15,16]. Thus, in order to commercially produce sea asparagus with desirable agricultural traits, it is important to understand how salt cultivation conditions affect productivity and chemical composition of these halophytes. In general, halophytes present salt-tolerance mechanisms based on ion transporters (triggered by ionic stress), salt compartmentalization in vacuoles, and organic osmolite production in the cytoplasm (for osmotic stress), adjustments of the photosynthetic apparatus and production of antioxidant molecules (for oxidative stress) [16–18]. Oxidative stress results from stomatal closure under salt exposure and inability of the plant to reduce electrons generated by photosynthesis in the light-dependent cycle, due to the lack of CO_2 molecules. This situation can lead-induce the reducing power (electrons) acts on other molecules generating reactive oxygen species (ROS). The photosynthetic pigments play an important role in preventing and combating oxidative stress in the photosystem (photooxidative stress). To avoid damage, chlorophyll b content may be favoured by a protective function of the photosystem due to its lower photo-oxidation rate compared to chlorophyll a [19]. Furthermore, carotenoids can also be activated in the extinction of electrons by transfer of excitation and harmless thermal dissipation (xanthophyll cycle). In addition, carotenoids act as antioxidant molecules against ROS generated if the intervention mechanisms are not efficient [18,20,21]. Besides that, several halophytes showed that their content of phenolic compounds are directly proportional to the intensity of salt stress [6,8,13,22] and the presence of these compounds is frequently linked to the action of scavengers against ROS [12,18].

Since 2010, the Laboratório de Biotecnologia de Halófitas ("BTH Lab", Instituto de Oceanografia, Universidade Federal do Rio Grande—FURG, Rio Grande, RS, Brazil) has been carrying out a breeding program that generated two distinctive inbred homozygous genotypes of southern Brazilian plants of *Salicornia neei* Lag., denominated BTH1 (prostate shoot growth and red phenotype at maturity) and BTH2 (decumbent shoot growth and green phenotype at maturity) [5,12,23]. *Salicornia neei* is a native South American species (previously named *Salicornia gaudichaudiana* Moq. and *Sarcocornia ambigua* (Michx.) M.A.Alonso & M.B.Crespo) and, besides their morphological and molecular differences [24], the two new genotypes show in their biomass distinct mineral and phenolic composition when irrigated with aquaculture saline effluent [5,12].

This study aimed at assessing the effects of salt stress exposure on growth, concentrations of photosynthetic pigments and phenolic compounds, and antioxidant capacity of the shoot biomass of two genotypes (BTH1 and BTH2) of *Salicornia neei*. *Salicornia neei* plants were evaluated through greenhouse and field trials for their growth performance and biochemical composition, and were also compared with traditional food plants.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Seeds of *S. neei* were obtained from the germplasm of the BTH Lab (FURG, Rio Grande, Brazil). Seeds of f3 progenies of BTH1 and BTH2 genotypes of *S. neei* were germinated at thermoperiod of 12 h (30 °C): 12 h (20 °C) [23]. Seedlings were transferred to styrofoam trays filled with a mixture (1:1) of an organic compound (Humosolo Vida®, Porto Alegre, Brazil) and fine beach sand and, 6 weeks later, to 50 cm^3 plugs with the same substrate. Seedlings were maintained for up to 38 weeks irrigated with tap water in a non-heated greenhouse located in FURG's Institute of Oceanography (32°4′43″ S, 52°10′3″ W) before the experiments.

2.2. Greenhouse Experiment

Thirty-four-week-old plants of the two *S. neei* genotypes were planted in a solution culture system. Individual plants were transferred to 50 cm^3 polyethylene plugs filled with washed fine beach sand. The plugs were placed into holes of a rack suspended over a 31 cm × 12 cm × 18 cm polyethylene trays, which were filled with modified full-strength Hoagland solution made with in deionized water with a final electrical conductivity (EC) of 1.7 dS m^{-1} and final concentration of Cl and Na of 0.09 mM and 0.06 mM, respectively. For each *S. neei* genotype, a total of six trays were used each holding 24 plugs (replicate plants). Five trays were subjected to the target salinity levels, and one tray was maintained as control (full strength Hoagland of EC = 1.7 dS m^{-1}; ~0.1 mM NaCl). Salinity levels were obtained by adding commercial marine salt Cisne® (Cabo Frio, Brazil) to full strength Hoagland solutions. The treatments consisted of control (full Hoagland without extra NaCl) and full Hoagland with final concentrations of 34.2, 85.5, 171, 513, and 769 mM NaCl. Salt treated plants were initially kept for two weeks in 34.2 mM NaCl (acclimation period) before the salt concentration of each treatment level was raised to full strength. Solutions were changed weekly and sand plugs kept close to the maximum holding capacity. The experiment had a randomized complete block design and it was carried out in non-heated greenhouse. The duration of the treatment was 50 days, during the austral summer (December 2014—January 2015). The average conditions inside greenhouse were: temperature 31.7 ± 3.2/20.1 ± 2.8 °C maximum/minimum; and irradiation 6.83 ± 2.22 MJ m^{-2} day^{-1}. Temperature inside the greenhouse was measured with a mercury thermometer, and a meteorological station of INMET (Institute National of Meteorology, Brazil), located in FURG campus, was used to obtain the values of irradiation. Pots were randomly re-located every two weeks.

For each plant, shoot height (cm) and branch number (only primary-first order branches) were quantified at the beginning (after acclimation period) and at the end of the experiment. Branch formation was calculated by the difference between initial and final values of branch number. Initial individual fresh (FW) and dry (DW) weights (g) were obtained from an extra lot of 10 plants kept in control solution. Dry weight was estimated by freeze-drying (lyophilized; −50 °C for 48 h) for better maintaining biomass quality. The succulence was defined as the water content in shoots and estimated by the percent difference between fresh and dry weight {[(FW − DW) × 100]/FW}. Relative shoot dry weight (RDW) was estimated to determine salinity effect in dry biomass production. For each salinity level, RDW was expressed as percentage of the average biomass of the control treatment (0.1 mM NaCl) biomass.

2.3. Field Experiment

Thirty-eight-week-old plants of *S. neei* BTH1 and BTH2 genotypes were planted in two separated 1.6 m × 3.5 m subplots established inside a larger plot (6.5 m × 3.5 m) localized in the Marine Aquaculture Station, FURG (32°12′19″ S; 52°10′45″ W). Plot design and local soil characteristics are described in Doncato and Costa [5]. Between November 2014 and April 2015 plants were watered every other day by filling up drainage ditches with 375 L of saline effluent from shrimp farm (*Litopenaeus vannamei*) with Biofloc Technology System, stocked with 87 shrimp m^{-2}. Water was

filtered through a net (mesh of 1 mm) to remove particulate suspended matter before being pumping into the experimental plot. Average environmental conditions (effluent, soil and meteorological) during field experiment are shown in Table 1.

Table 1. Average (± standard deviation) conditions of effluent (salinity, pH, dissolved oxygen—DO, nitrate, total ammonium nitrogen—TAN and phosphate), soil (moisture and electrical conductivity—EC) and meteorological data (maximum and minimum temperatures, solar radiation, and accumulated rainfall) during the field experiment.

Effluent and Environment Parameters	
Salinity (mM NaCl)	213 ± 17.0
pH	8.65 ± 0.19
DO (mg L^{-1})	7.59 ± 0.58
Nitrate (mg L^{-1})	<0.03
TAN (mg L^{-1})	0.15 ± 0.62
Phosphate (mg L^{-1})	0.30 ± 0.24
Soil	
Moisture (%) *	12.4 ± 5.4
EC (dS m^{-1}) *	14.3 ± 5.6
Meteorological **	
T max. (°C)	24.5 ± 2.6
T min. (°C)	23.3 ± 2.7
Radiation (MJ m^{-2} day^{-1})	19.8 ± 6.97
Rainfall (mm)	340

*, The plot soil moisture and EC were determined by Doncato and Costa [5]. **, Daily meteorological data were obtained from the INMET station (Brazilian National Institute of Meteorology) located on the FURG campus (32°04′43″ S; 52°10′03″ W), approximately 20 km from the field site.

After 154 days (22 weeks) of field cultivation, plants were cut just above ground level, washed to remove soil and excess water was removed from the shoots using an absorbent paper towel. Shoot heights were measured with a ruler (mm) and branch numbers were quantified. Shoots were weighed on a precision scale to determine fresh weights. Five plants of each *S. neei* variety were randomly chosen, rinsed with distilled water and frozen at −20 °C. These samples were lyophilized (−48 °C during 48 h), dry weight determined with a precision scale, and biomass ground in knife mills. Subsequently, succulence was estimate according to equation previously presented. Initial shoot fresh and dry weights were obtained from an extra lot of 10 plants collected at the planting date.

2.4. Plant Metabolites and Free-Radical Scavenging Capacity of Shoot Extracts

Plant of both *S. neei* genotypes cultivated at different salinity levels inside the greenhouse and at the field plot irrigated with saline effluent had their shoot metabolites quantified. Chemical analyses were performed in triplicate with the homogeneous fresh sample freeze-drying shoots.

2.4.1. Photosynthetic Pigments

Photosynthetic pigments were determined only for shoot samples from the greenhouse experiment following the method recommended by Mendes et al. [25]. Samples were sonicated for 5 min in an ice-water bath, placed at −20 °C for 1 h, and then centrifuged at 1100 *g* for 5 min at 3 °C Supernatants were filtered through fluoropore PTFE membrane filters (0.2 µm pore size; Merck Millipore Ltd., Billerica, MA, USA), diluted (1 mL of sample was mixed with 0.40 mL of ultrapure water) in 2-mL amber glass sample vials, and immediately placed in the HPLC cooling rack (4 °C). The extracted pigments were analysed in a Shimadzu HPLC (Shimadzu Corporation, Kyoto, Japan) using monomeric C8 column (SunFire; 15 cm, 4.6 mm, 3.5 µm) and the following mobile phases: (A) methanol: acetonitrile: aqueous pyridine solution (0.25 M, pH adjusted to 5.0 with acetic acid) (50:25:25, *v*/*v*/*v*), and (B) methanol: acetonitrile: acetone (20:60:20, *v*/*v*/*v*). The solvent gradient with

a flow rate of 1 mL min^{-1}, with an injection volume of 100 µL, and 40 min total run. The HPLC system was calibrated with pigment standards, namely, chlorophyll a, chlorophyll b, β-carotene, lutein, neoxanthin, violaxanthin and zeaxanthin from DHI (Institute for Water and Environment, Denmark). Pigment quantifications were done in triplicates, and results were expressed in µg g^{-1} dry weight.

To evaluate the light harvesting and photo-protection mechanisms, the ratios of chlorophyll a:b and the total chlorophyll to total carotenoids, as well as the de-epoxidation state of xanthophylls (DES), were calculated [13,18].

2.4.2. Phenolic Compounds and Antioxidant Capacity

Extractions of phenolic compounds were carried out in triplicate by ultrasound-assisted extraction and free phenolic compounds (FPC) were measured by the Folin-Ciocalteau method, using a spectrophotometer at 750 nm (Biospectro, SP-22, Brazil) [26]. The concentration of FPCs was estimated using a standard curve of gallic acid (2.2 to 22 µg mL^{-1}) and free phenolic content was expressed as milligram of gallic acid equivalents (GAE) per gram of sample dry weight (mg GAE g^{-1} dw). Free flavonoid compounds (FFC) were measured according to Gajula et al. [27]. 0.11 mL of 80% ethanol was added in 0.16 mL of extract, followed by the addition of 0.08 mL of $AlCl_3$ solution (5% w/v). After thirty minutes, the spectrophotometric determination was performed at 405 nm (Reader TP thermoplate nm). FFCs were estimated using a standard curve of quercetin (0.62 to 10 µg mL^{-1}) and free flavonoid content was expressed as milligram of quercetin equivalents (QE) per gram of sample dry weight (mg QE g^{-1} dw).

The identification and quantification of phenolic acids and quercetin in the extracts were carried out by HPLC-UV-Vis (Shimadzu LC-10, Tokyo, Japan), equipped with a Discovery Bio Wide Pore C18 column (5 µm, 25 cm × 4.6 mm) (adapted of the Scaglioni et al. [28]). The extract was mixed with mobile phase (1:1) and centrifuged at 1400 *g* (Eppendorf Centrifuge 5804 R, Hamburg, Germany) prior to injection. The HPLC-UV-Vis operated at a flow rate of 0.7 mL min^{-1}, at 35 °C, using a gradient mobile phase consisting of methanol and acidified ultrapure water (glacial acetic acid 1%) (0 min = 30%; 5 min = 40%; 10 min = 50%; 15 min = 60%, and 20 min = 30% methanol; v/v) for 30 min. The wavelength utilized was 280 nm, changing to 320 nm between 14 and 25 min. The contents of phenolic acids and quercetin were expressed as microgram per gram of dry weight sample (µg g^{-1} dw).

The free-radical scavenging capacity of each extract was investigated against DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical, and estimated according to the modified method of Nicklisch and Waite [29]. 10 µL of ethanolic DPPH solution (0.80 mg mL^{-1}) were added to 190 µL of extract in different concentrations (10, 7 and 5 mg plant dw mL^{-1}). Absorbance was measured at 490 nm (TP Reader NM Thermoplate) at intervals of 10 min for 1 h. Measurements started immediately after mixing the solutions.

Analyses were performed in triplicates and DPPH inhibition percentage (%I_{DPPH}) was estimated. For each salinity level of the greenhouse experiment and also for field cultivated plants the plant extract concentration necessary to produce 50% I_{DPPH} (IC_{50}), was determined, estimated by linear regressions (extract concentrations vs. %I_{DPPH}).

2.5. Statistical Analysis

All results were expressed as means and standard deviations. Two-way ANOVA was used to evaluate the effect of salinity and genotype identity (and their interaction) on growth, concentrations of photosynthetic pigments, phenolic compounds and antioxidant activity of *S. neei* shoot biomass. The ANOVAs were followed with a comparison by Tukey's HSD test at $p < 0.05$ significance level. Normality of residues and homoscedasticity were tested by Shapiro-Wilks and Bartlett test, respectively. To accomplish ANOVA assumptions of normality and homoscedasticity, greenhouse experiment values of branch number, syringic acid and violaxanthin contents, and DES were transformed by square root. Lutein, neoxanthin and sum of carotenoids were transformed by ln(x). Succulence and zeaxanthin content was transformed by x^7 and 1/x, respectively. For each genotype, Pearson linear correlation

was used to evaluate relationships among antioxidant activity (50% I_{DPPH}—IC50) and the contents of metabolites (pigments and phenolic compounds) in shoots. Field data of growth, shoot contents of metabolite and antioxidant activity of the two *S. neei* genotypes were compared by Student's *t*-test at $p < 0.05$ significance level. All statistical analyses were performed in R software (3.5.0, R Core Team, Vienna, Austria), and values of the limit of detection were used instead of "zero" when a metabolic parameter had its quantity not detected by the analytical method.

3. Results

3.1. Responses of S. neei Genotypes to Different Salinity Levels (Greenhouse Experiment)

3.1.1. Growth Responses

The average initial shoot height, branch number, fresh and dry weights of *S. neei* genotypes were 11.4 ± 3.6 cm, 2.2 ± 3.3, 0.30 ± 0.20 g and 0.07 ± 0.02 g, respectively. Except for shoot branch number (3.5 ± 3.7 and 0.9 ± 2.2 branches per shoot for BTH1 and BTH2, respectively), the initial values of plant attributes did not show statistical differences among salinity levels and between plant genotypes. After fifty days of cultivation in different salinities, no plant death was observed for *S. neei* genotypes. Shoot height did not significantly differ between *S. neei* genotypes and among salinity levels. Final shoot branch number of BTH1 genotype was twice larger than BTH2 plants but neither genotype had the branch number significantly affected by salinity (Table 2).

Salicornia neei genotypes showed similar final shoot fresh and dry weights, but only shoot fresh weight was significantly affected by salinity. Genotypes showed different biomass responses to salinity stress (significant interaction genotype X salinity), being BTH1 plants grown at 86 mM NaCl, heavier than those subject to higher salinity (769 mM NaCl), whereas BTH2 fresh weight was not affected by salinity (Tukey test; Table 2). Different statistical results between shoot fresh and dry weights may be explained by observed inability of BTH1 to hold its high shoot succulence (average values 75–78% of water in salinities below 171 mM NaCl) in salinities equal and higher than 513 mM NaCl (average values drop to 52–59%). BTH2 plants showed higher global average succulence not statically affected by salinity (succulence ranged between 67% and 80%). This distinct behaviour for succulence was detected by a significant genotype X salinity interaction (Table 2).

Salicornia neei genotypes showed their largest average biomass at low salinity levels (BTH1 at 86 mM NaCl and BTH2 at 34 mM NaCl). Plants of BTH1 and BTH2 genotypes exposed to these low salinities showed relative biomass (RDW) 129% and 119% of the average shoot biomass of their plants grown in the absence of salinity, respectively.

Table 2. Averages (± standard deviation) of height (cm), branch number, fresh weight (g), dry weight (g), relative shoot dry weight (RDW; % of the average biomass of plants grown at 0 mM NaCl) and succulence (%) of *Salicornia neei* genotypes (BTH1 and BTH2) after 50 days of cultivation in greenhouse at different salinities (0.1, 34, 86, 171, 513 and 769 mM NaCl).

Genotype	Salinity	Height		Branch Number		Fresh Weight		Dry Weight		RDW	Succulence	
BTH1	0.1	15.3 ± 1.7 ^{a}		8.2 ± 5.6 ^{ab}		0.60 ± 0.22 ^{abc}		0.15 ± 0.05 ^{a}		100	75.2 ± 3.3 ^{ab}	
	34	13.6 ± 2.6 ^{a}		5.6 ± 4.3 ^{abc}		0.52 ± 0.11 ^{abc}		0.11 ± 0.03 ^{a}		75.7	78.3 ± 4.2 ^{a}	
	86	15.4 ± 3.6 ^{a}		8.8 ± 5.6 ^{a}		0.85 ± 0.29 ^{a}		0.19 ± 0.07 ^{a}		129	77.8 ± 4.7 ^{ab}	
	171	14.8 ± 2.2 ^{a}		5.5 ± 3.6 ^{abc}		0.43 ± 0.15 ^{bc}		0.11 ± 0.03 ^{a}		76.5	73.1 ± 6.0 ^{abc}	
	513	13.8 ± 2.4 ^{a}		5.8 ± 2.5 ^{abc}		0.32 ± 0.14 ^{bc}		0.13 ± 0.05 ^{a}		88.1	59.1 ± 5.7 ^{c}	
	769	14.3 ± 2.7 ^{a}		6.9 ± 4.5 ^{ab}		0.28 ± 0.18 ^{c}		0.12 ± 0.04 ^{a}		79.2	52.5 ± 15.1 ^{c}	
BTH2	0.1	15.0 ± 3.6 ^{a}		1.3 ± 1.7 ^{c}		0.55 ± 0.23 ^{abc}		0.11 ± 0.05 ^{a}		100	79.5 ± 2.1 ^{a}	
	34	15.6 ± 2.6 ^{a}		3.2 ± 4.6 ^{bc}		0.66 ± 0.29 ^{ab}		0.13 ± 0.07 ^{a}		119	80.2 ± 1.9 ^{a}	
	86	16.1 ± 2.8 ^{a}		2.7 ± 3.8 ^{bc}		0.60 ± 0.20 ^{abc}		0.13 ± 0.04 ^{a}		116	78.1 ± 4.1 ^{ab}	
	171	15.2 ± 2.5 ^{a}		3.0 ± 3.6 ^{abc}		0.50 ± 0.22 ^{bc}		0.12 ± 0.03 ^{a}		105	75.5 ± 5.1 ^{ab}	
	513	15.3 ± 3.5 ^{a}		3.8 ± 4.8 ^{abc}		0.41 ± 0.30 ^{bc}		0.11 ± 0.04 ^{a}		103	66.9 ± 9.1 ^{bc}	
	769	15.3 ± 2.7 ^{a}		4.7 ± 4.0 ^{abc}		0.55 ± 0.21 ^{abc}		0.12 ± 0.03 ^{a}		111	75.2 ± 8.6 ^{ab}	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*		*F*	*p*
Genotype (G)		3.21	0.757	35.0	***<0.001***	2.88	0.335	2.17	0.143	-	17.2	***<0.001***
Salinity (S)		0.46	0.500	1.76	0.187	36.2	***<0.001***	0.61	0.437	-	49.1	***<0.001***
Interaction (G × S)		0.18	0.676	2.42	0.122	12.3	***0.016***	1.68	0.197	-	7.84	***0.006***

Different lowercase letters (within a column) represent significant differences between the averages ($p < 0.05$), according to the Tukey's HSD test.

3.1.2. Pigments and Photooxidative Stress Indices

All average concentrations of pigments in BTH1 genotype shoots were higher than those observed in BTH2 shoots (Table 3). Chlorophylls' contents were negatively affected by salinity in both *S. neei* genotypes. BTH1 had the highest averages of chlorophylls a (190 μg g^{-1} dw) and b (103 μg g^{-1} dw) in control treatment (0.1 mM NaCl) and lowest values in 513 mM NaCl (a = 78.0 μg g^{-1} dw; b = 40.4 μg g^{-1} dw), whereas highest and lowest averages of BTH2 genotype were observed in 34 mM NaCl (a = 152 μg g^{-1} dw; b = 67.1 μg g^{-1} dw) and 769 mM NaCl (a = 41.6 μg g^{-1} dw; b = 22.2 μg g^{-1} dw), respectively. *Salicornia neei* genotypes showed different chlorophyll *a*:*b* ratios (higher in BTH2; F = 15.3, $p < 0.001$) and chlorophyll b percentage in the total chlorophyll content (higher in BTH1; F = 19.8, $p < 0.001$), and both parameters showed distinct behaviour under salinity stress (significance interaction genotype X salinity; chlorophyll *a*:*b* ratio F = 10.9 and $p = 0.002$; chlorophyll b percentage F = 5.3 and $p = 0.002$). BTH2 chlorophyll *a*:*b* ratio decreased and chlorophyll b percentage raised with increasing salinities, whereas BTH1 values of these parameters were not affected by salinity (Tukey test; Figure 1A,B).

Zeaxanthin content values were not detected and the de-epoxidation state of xanthophylls (DES) was not estimated in BTH2 genotype, but both parameters were positively affected by salinity in BTH1 (F = 16.4, $p < 0.001$). BTH1 zeaxanthin averages ranged between 0.39 μg g^{-1} dw (at 34 mM NaCl) and 0.88 μg g^{-1} dw (at 86 mM NaCl), and the de-epoxidation state (DES) ranged between 0.09 ± 0.03 (0.1 mM NaCl) and 0.43 ± 0.09 (at 513 mM NaCl) (Figure 1C). The contents of other xanthophylls (lutein, neoxanthin and violaxanthin) in shoots of both genotypes were also reduced with increasing salinities (Table 3) Maximum xanthophyll contents were observed between 0.1 (BTH1) and 34 (BTH2) mM NaCl, and minimum values between 513 (BTH1) and 769 (BTH2) mM NaCl. The highest β-carotene average value was observed in BTH1 shoots at 0.1 mM NaCl (1.98 μg g^{-1} dw), which fluctuated but was not statically affected by increasing salinities. Small concentrations of this carotenoid (0.13 μg g^{-1} dw) in BTH2 shoots were detected only in unsalted control plants and under 34 mM NaCl (0.43 μg g^{-1} dw). The ratio total chlorophyll:total carotenoids was not affected by salinity in both *S. neei* genotypes, but BTH1 shoots presented average ratio higher than that of BTH2 (Table 3).

3.1.3. Phenolic Compounds and Antioxidant Activity

Shoots of BTH1 and BTH2 showed high and similar FPC contents (Table 4) not affected by salinity. FPC averages ranged from 11.5 to 15.7 mg GAE g^{-1} dw in BTH1, and from 12.3 to 13.9 mg GAE g^{-1} dw in BTH2. Concentrations of FFC, syringic acid and vanillin were higher in BTH1 shoots and quercetin content was higher in BTH2 shoots. FFC content was not affected by salinity. Salinity negatively affected the contents of vanillin and syringic acid (only BTH1 genotype). Contrariwise, quercetin content was positively affected by salinity increment and both genotypes showed lower averages at 86 mM NaCl (BTH1 = non detected; BTH2 = 5.45 μg g^{-1} dw) and higher values at 769 mM NaCl (BTH1 = 4.56 μg g^{-1} dw; BTH2 = 11.6 μg g^{-1} dw).

BTH1 shoot extract presented antioxidant activity higher than that of BTH2, but IC_{50} was not affected by salinity. Higher antioxidant capacities (lower IC_{50} values) of BTH1 (IC_{50} = 4.37 mg dw mL^{-1}) and BTH2 (IC_{50} = 5.31 mg dw mL^{-1}) were observed at 86 and 171 mM NaCl, respectively. IC_{50} was correlated with only FPC ($r = -0.69$, $p < 0.05$) and FFC contents ($r = -0.56$, $p < 0.05$) in BTH1 genotype, thus antioxidant activity increase with larger contents of these compounds. For BTH2, antioxidant activity was not correlated with any metabolites evaluated.

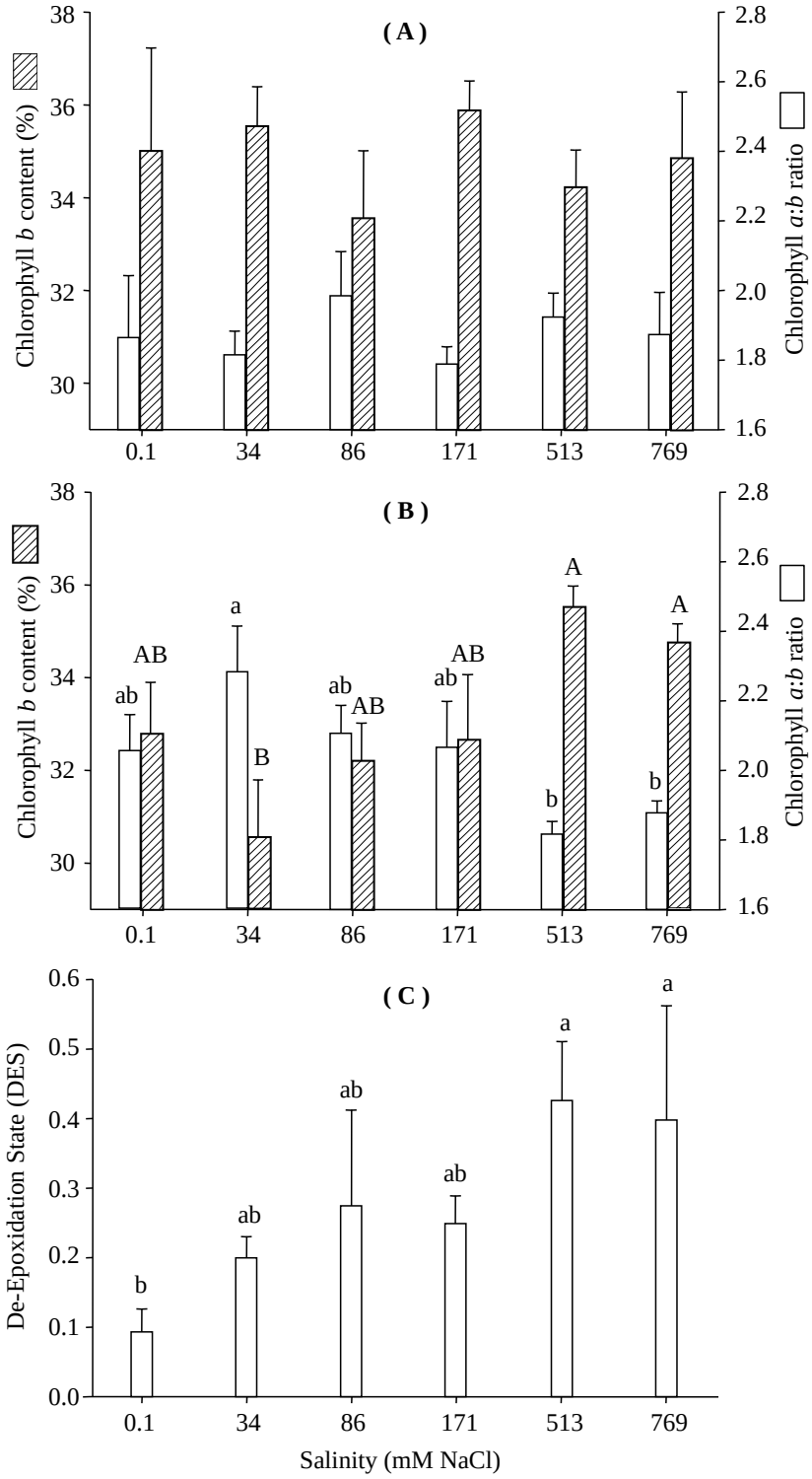

Figure 1. Photo-protective mechanisms of *Salicornia neei* genotypes under increasing salinities in greenhouse experiment. Chlorophyll *a*:*b* ratio and percentage content of chlorophyll b of BTH1 (**A**) and BTH2 genotypes (**B**). De-epoxidation state of xantophylls (DES) of BTH1 genotype (**C**). Different lowercase letters above hollow bars denote significant (Tukey HSD test; $p < 0.05$) differences of Chlorophyll *a*:*b* ratio and DES among salinity levels, while different capital letters above dashed bars denote significant differences of percentage of chlorophyll b among salinity levels.

Table 3. Averages (± standard deviation) of photosynthetic pigment contents (in μg g^{-1} dw) of *Salicornia neei* genotypes (BTH1 and BTH2) after 50 days of cultivation in greenhouse at different salinities (0.1, 34, 86, 171, 513 and 769 mM NaCl).

Genotype	Salinity	Chlorophyll a	Chlorophyll b	β-Carotene §	Lutein	Neoxanthin	Violaxanthin	Zeaxanthin §	Σcar:Σchl
BTH1	0.1	190 ± 6.02 ^{a}	103 ± 11.9 ^{a}	1.98 ± 0.43 ^{a}	28.6 ± 6.02 ^{a}	7.20 ± 2.33 ^{a}	4.46 ± 0.97 ^{a}	0.44 ± 0.09 ^{a}	0.14 ± 0.02 ^{a}
	34	85.0 ± 8.26 ^{abc}	46.7 ± 2.95 ^{bc}	0.47 ± 0.05 ^{a}	13.3 ± 0.49 ^{abc}	2.98 ± 0.88 ^{abc}	1.56 ± 0.13 ^{b}	0.39 ± 0.10 ^{a}	0.14 ± 0.01 ^{a}
	86	155 ± 27.9 ^{ab}	78.3 ± 14.6 ^{ab}	0.99 ± 0.41 ^{a}	20.0 ± 4.55 ^{ab}	4.55 ± 1.65 ^{ab}	2.29 ± 0.41 ^{ab}	0.88 ± 0.51 ^{a}	0.12 ± 0.01 ^{a}
	171	103 ± 34.7 ^{abc}	57.7 ± 20.7 ^{abc}	0.76 ± 0.29 ^{a}	14.4 ± 4.74 ^{abc}	3.98 ± 1.13 ^{abc}	1.76 ± 0.41 ^{b}	0.57 ± 0.03 ^{a}	0.14 ± 0.01 ^{a}
	513	78.0 ± 33.1 ^{bc}	40.4 ± 16.7 ^{bc}	0.53 ± 0.17 ^{a}	9.00 ± 2.74 ^{bc}	2.13 ± 0.51 ^{bc}	1.14 ± 0.35 ^{bc}	0.82 ± 0.09 ^{a}	0.12 ± 0.02 ^{a}
	769	86.0 ± 22.5 ^{abc}	45.6 ± 9.32 ^{bc}	0.76 ± 0.15 ^{a}	11.4 ± 2.14 ^{abc}	2.54 ± 0.43 ^{abc}	1.43 ± 0.72 ^{c}	0.86 ± 0.24 ^{a}	0.13 ± 0.02 ^{a}
BTH2	0.1	87.3 ± 21.3 ^{abc}	42.9 ± 12.5 ^{bc}	0.13 ± 0.22	12.6 ± 3.92 ^{abc}	2.93 ± 1.08 ^{abc}	1.25 ± 0.25 ^{b}	nd	0.13 ± 0.01 ^{a}
	34	152 ± 5.70 ^{ab}	67.1 ± 6.49 ^{abc}	0.43 ± 0.37	17.2 ± 2.60 ^{abc}	3.52 ± 1.51 ^{abc}	1.28 ± 0.21 ^{b}	nd	0.10 ± 0.02 ^{a}
	86	66.5 ± 12.0 ^{bc}	31.5 ± 4.46 ^{bc}	nd	8.56 ± 1.01 ^{bc}	2.13 ± 0.22 ^{bc}	0.77 ± 0.22 ^{bc}	nd	0.12 ± 0.01 ^{a}
	171	61.8 ± 3.53 ^{bc}	29.9 ± 0.62 ^{bc}	nd	8.22 ± 0.58 ^{bc}	1.94 ± 0.33 ^{bc}	0.76 ± 0.19 ^{bc}	nd	0.12 ± 0.01 ^{a}
	513	41.9 ± 12.7 ^{c}	23.2 ± 7.48 ^{c}	nd	6.80 ± 3.21 ^{c}	1.58 ± 0.99 ^{c}	0.25 ± 0.22 ^{cd}	nd	0.13 ± 0.03 ^{a}
	769	41.6 ± 6.01 ^{c}	22.2 ± 3.60 ^{c}	nd	6.13 ± 1.00 ^{bc}	1.54 ± 0.27 ^{bc}	nd ^{d}	nd	0.12 ± 0.01 ^{a}

	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Genotype (G)	11.0	***0.002***	18.0	***<0.001***	-	-	18.3	***<0.001***	16.9	***<0.001***	60.8	***<0.001***	-	-	6.46	***0.016***
Salinity (S)	16.8	***<0.001***	15.6	***<0.001***	2.61	0.126	25.1	***<0.001***	18.6	***<0.001***	50.8	***<0.001***	8.94	***0.009***	0.16	0.694
Interaction (G × S)	0.01	0.936	0.25	0.624	-	-	0.04	0.840	0.03	0.854	4.49	***0.042***	-	-	1.91	0.177

Different lowercase letters (within a column) represent significant differences between the averages ($p < 0.05$), according to the Tukey's HSD test. §, Oneway ANOVA was applied to evaluate salinity effect on BTH1 genotype because of the occurrence of non-detected (nd) values for BTH2 genotype shoots in most of salinities levels tested. Σcar:Σchl is the total carotenoids to total chlorophyll ratio.

Table 4. Averages (± standard deviation) of phenolic compounds' contents and antioxidant capacity of shoots (dry weight) of *Salicornia neei* genotypes (BTH1 and BTH2) after 50 days of cultivation in greenhouse at different salinities (0.1, 34, 86, 171, 513 and 769 mM NaCl).

Genotype	Salinity	FPC (mg GAE g^{-1})	FFC (mg QE g^{-1})	Quercetin (μg g^{-1})	Syringic Acid (μg g^{-1}) §	Vanillin (μg g^{-1})	IC50 (mg mL^{-1})
BTH1	0.1	14.8 ± 1.80 ^{a}	0.67 ± 0.20 ^{a}	3.82 ± 0.48 ^{bcd}	1.44 ± 0.58 ^{a}	5.87 ± 1.39 ^{a}	4.78 ± 0.82 ^{a}
	34	14.3 ± 1.04 ^{a}	0.76 ± 0.06 ^{a}	1.07 ± 1.81 ^{cd}	1.48 ± 0.79 ^{a}	5.22 ± 0.68 ^{ab}	5.10 ± 1.32 ^{a}
	86	15.7 ± 0.65 ^{a}	0.82 ± 0.16 ^{a}	nd ^{d}	1.11 ± 0.27 ^{ab}	5.39 ± 0.94 ^{ab}	4.37 ± 1.79 ^{a}
	171	11.5 ± 2.64 ^{a}	0.63 ± 0.15 ^{a}	3.62 ± 3.11 ^{bcd}	0.88 ± 0.39 ^{ab}	4.78 ± 0.82 ^{ab}	6.00 ± 0.59 ^{a}
	513	11.7 ± 1.05 ^{a}	0.50 ± 0.01 ^{a}	4.36 ± 0.52 ^{bcd}	0.35 ± 0.29 ^{b}	4.09 ± 0.37 ^{ab}	5.66 ± 0.30 ^{a}
	769	14.3 ± 0.14 ^{a}	0.59 ± 0.15 ^{a}	4.56 ± 0.34 ^{bcd}	0.23 ± 0.20 ^{b}	4.15 ± 0.27 ^{ab}	4.75 ± 0.54 ^{a}
BTH2	0.1	13.7 ± 1.00 ^{a}	0.43 ± 0.12 ^{a}	6.43 ± 0.28 ^{abcd}	0.23 ± 0.24	4.96 ± 0.70 ^{ab}	5.88 ± 1.13 ^{a}
	34	13.9 ± 0.96 ^{a}	0.51 ± 0.08 ^{a}	5.84 ± 0.83 ^{abcd}	0.14 ± 0.14	4.33 ± 0.22 ^{ab}	5.86 ± 1.17 ^{a}
	86	12.4 ± 1.61 ^{a}	0.40 ± 0.01 ^{a}	5.45 ± 0.62 ^{abcd}	nd	4.29 ± 0.10 ^{ab}	6.65 ± 1.16 ^{a}
	171	13.9 ± 0.94 ^{a}	0.64 ± 0.23 ^{a}	7.32 ± 4.85 ^{abc}	nd	4.60 ± 1.16 ^{ab}	5.31 ± 1.06 ^{a}
	513	12.3 ± 1.04 ^{a}	0.44 ± 0.19 ^{a}	9.54 ± 2.73 ^{ab}	nd	4.33 ± 0.27 ^{ab}	6.50 ± 0.57 ^{a}
	769	12.7 ± 1.20 ^{a}	0.47 ± 0.12 ^{a}	11.6 ± 5.19 ^{a}	nd	3.54 ± 0.35 ^{b}	5.51 ± 0.67 ^{a}

	F	*p* value	*F*	*p* value	*F*	*p* value	*F*	*p* value	*F*	*p* value	*F*	*p* value
Genotype (G)	1.09	0.305	13.8	***<0.001***	36.5	***<0.001***	-	-	6.26	***0.018***	5.95	***0.021***
Salinity (S)	2.60	0.117	2.61	0.116	16.8	***<0.001***	28.1	***<0.001***	16.0	***<0.001***	0.00	0.974
Interaction (G × S)	0.01	0.929	2.02	0.164	1.78	0.192	-	-	1.05	0.313	0.12	0.730

Different lowercase letters (within a column) represent significant differences between the averages ($p < 0.05$), according to the Tukey's HSD test. §, Oneway ANOVA was applied to evaluate salinity effect on BTH1 genotype because of the occurrence of non-detected (nd) values for BTH2 genotype shoots in most of salinities levels tested. FPC—Free Phenolic Compounds; FFC—Free Flavonoids Compounds; GAE—Gallic Acid Equivalent; QE—Quercetin Equivalent.

3.2. Growth, Biomass Production and Shoot Composition of S. neei Genotypes under Saline Effluent Irrigation in Field Conditions

Salicornia neei genotypes had initial heights (BTH1 = 19.8 ± 1.54 cm; BTH2 = 24.2 ± 1.71 cm), fresh (BTH1 = 26.4 ± 0.35 g; BTH2 = 27.3 ± 0.38 g) and dry weight (BTH1 = 6.23 ± 0.99 g; BTH2 = 9.04 ± 1.10 g) significantly different for the two genotypes. After 154 days of irrigation with saline shrimp farm effluent in the field, no plant death was observed. Contrasting with the results of the greenhouse experiment, BTH2 genotype, in general, showed better performance than BTH1 (Table 5). Average values of biomass and succulence of BTH2 plants were higher than those of BTH1, resulting in superior BTH2 yield (5.74 ton fw h^{-1}; BTH1 = 2.05 ton fw hA^{-1}). No statistical difference was found between genotypes for shoot height and branch number.

Table 5. Average values (± standard deviation) of growth parameters, shoot phenolic compounds and antioxidant capacity of *Salicornia neei* genotypes (BTH1 and BTH2) after 154 days growth in field plot irrigated with saline shrimp farm effluent.

			Student's *t*-Test	
	BTH1	**BTH2**	***t***	***p***
Growth parameters				
Height (cm)	40.6 ± 6.81	51.6 ± 9.12	−2.16	0.065
Branch number	52.4 ± 11.8	52.8 ± 6.53	−0.07	0.949
Fresh Weight (g)	56.4 ± 10.2	158 ± 31.2	−6.92	***0.001***
Dry Weight (g)	14.9 ± 1.92	25.4 ± 5.40	−4.10	***0.009***
Succulence (%)	72.9 ± 5.97	84.0 ± 0.48	−4.30	***0.008***
Shoot yield (ton fw ha^{-1})	2.05	5.74		
Phenolic compounds				
FPC (mg GAE g^{-1} dw)	13.1 ± 1.84	22.2 ± 2.38	−5.27	***0.007***
FFC (mg QE g^{-1} dw)	0.54 ± 0.12	5.06 ± 1.83	−4.26	***0.005***
Quercetin (μg g^{-1} dw) ‡	nd	14.8 †		
Protocatechuic acid (μg g^{-1} dw)	4.91 ± 0.06	10.0 ± 0.64	−11.2	***0.007***
Chlorogenic acid (μg g^{-1} dw)	1.67 ± 1.01	3.46 ± 3.93	−0.75	0.519
Gallic acid (μg g^{-1} dw)	0.38 ± 0.10	0.64 ± 0.24	−1.41	0.263
Caffeic acid (μg g^{-1} dw) ‡	nd	1.21 ± 0.43		
Syringic acid (μg g^{-1} dw)	6.82 ± 2.28	1.51 ± 0.37	3.25	***0.031***
Vanillin (μg g^{-1} dw)	5.24 ± 0.78	2.73 ± 0.87	3.03	***0.039***
Ferulic acid (μg g^{-1} dw) ‡	0.39 ± 0.02	nd		
Antioxidant capacity				
IC_{50} (mg dw mL^{-1})	14.3 ± 7.11	5.41 ± 2.28	2.05	0.154

FPC—Free Phenolic Compounds; FFC—Free Flavonoid Compounds; GAE—Gallic Acid Equivalent; QE—Quercetin Equivalent; Nd—non detected; IC_{50}—estimated shoot mass per mL necessary to inhibit 50% of DPPH radicals. †, Concentration was detected in one sample only; ‡, Statistical analysis not performed.

The average total contents of FPC (22.2 mg GAE g^{-1} dw) and FFC (5.06 mg QE g^{-1} dw) in BTH2 shoots were 2- and 10-fold higher than those in BTH1 shoots, respectively (Table 5). Higher contents of phenolic and a larger number of phenolic acids were quantified in the field cultivated plants. BTH1 showed higher content of vanillin, syringic and ferulic acids, whereas BTH2 had higher concentration of quercetin, as well as caffeic, chlorogenic, gallic and protocatechuic acids. Although it was necessary a 2.6-fold smaller amount of BTH2 shoot mass (IC_{50} = 5.41 mg mL^{-1}) than in BTH1 mass (IC_{50} = 14.3 mg mL^{-1}) to inhibit 50% of DPPH radicals, no statistical difference was found between the antioxidant activities of *S. neei* genotypes.

4. Discussion

4.1. Responses of S. neei Genotypes to Different Salinity Levels

The two new genotypes of *Salicornia neei* behaved as constitutive halophytes, showing maximum growth under moderate salinities rather than under the non-saline control treatment. This response, observed in other succulent extreme halophytes, results from a better homoeostasis under salt conditions [1]. Furthermore, several growth parameters (height, branch number and shoot dry weight) and biochemical characteristics (FPC, FFC and antioxidant activity) showed only minor and non-significant differences among the salinity levels tested. Our results indicate that, in order to sustain growth under salt stress, *S. neei* genotypes regulate their physiological mechanisms related to succulence, production of photoprotective pigments, and antioxidant metabolites.

Maximum water content in the shoot biomass of *S. neei* genotypes cultivated in greenhouse was 78–80%, on average. High succulence of *S. neei* genotypes was observed between 0.1 and 171 mM NaCl; above this range succulence decreases for BTH1, but did not significantly changed for BTH2. Redondo-Gómez et al. [17] and García-Caparrós et al. [30] did not find variation in root and shoot water content of *Sarcocornia fruticosa* grown under greenhouse at salinity levels ranging between 0–1030 mM NaCl and 10–300 mM NaCl, respectively. These above cited responses contrast with the more common mechanism used by succulent halophytes to cope with saline stress, which is water absorption for metabolic functions by salt ion compartmentation (e.g., Na^+ and Cl^-) into the vacuole, allowing maintenance of osmotic and ionic balances between intra and extracellular media, including salt dilution in the vacuole [1,16]. The decrease of succulence in BTH1 genotype and the maintenance of succulence by BTH2 genotype at high salinities were both associated with visually detected epithelial lignification of their shoots. This lignification response was also observed by other authors [16,31] who suggested that, at a toxic ionic vacuolar concentrations, some halophytes can reduce water loss by lignifying shoot tissue instead of increasing succulence.

Lignin content was not directly evaluated in this work, but phenolic acid data may be used as proxies for lignin metabolism. Syringic acid and vanillin are generated by lignin degradation in industrial plants (vanillin production) [32] and in experimental tests (insoluble bound forms with lignin and carbohydrates) [33]. Thus, the tissue content of these two compounds may be directly related to lignin biosynthesis. Regarding shoot succulence, vanillin and syringic acid content decreased in *S. neei* shoots with the increment of salinity, showing lower values in salinities above than 171 mM NaCl (Tables 2 and 3). Thus, under salt stress *S. neei* (particularly BTH1 genotype) seems to consume phenolic acids (vanillin and syringic acid) for lignin biosynthesis. Furthermore, the lignification is activated in *Salicornia europaea* L. shoots exposed to 200 mM NaCl (or higher), and this process can be associated with a decreased concentration of photosynthetic pigments [22].

The flavonoid quercetin may also play an important role in the succulence-lignification adjustment to salt stress of *S. neei*. In both genotypes studied, quercetin content decreased from 0.1 to 86 mM NaCl. This behaviour changed in plants exposed to solutions with more than 171 mM NaCl, and quercetin shoot concentration continuously increased up to the highest salinity tested (769 mM NaCl). Quercetin and its derivatives perform multiple functions in plants, such as neutralization of ROS (associated with stomata closure), inhibition of the auxin efflux facilitators, and they also can contribute to lignification [20,34]. Apparently, at low salinity, quercetin content in *S. neei* (but particularly BHT2 variety) decreases in favour of plant growth (increased height, fresh and dry biomass, and succulence). However, these initial physiological adjustments (e.g., succulence) cannot be maintained by plants exposed to 171 mM NaCl (or higher salinities), making quercetin production necessary for the antioxidant action and to contribute in plant lignification. In the greenhouse experiment, both genotypes showed highest FPC, FFC and antioxidant capacity at low salinities (34–86 mM NaCl); however, the BTH1 genotype had a higher but much more salt-sensitive contents of all phenolic metabolites studied (except quercetin), which fell abruptly at high salt concentrations. Although not statistically salt-affected, the antioxidant capacity of BTH1 genotype was positively correlated

with the total content of phenolic compounds (FPC and FFC). This correlation was also observed by Costa et al. [12] in the *S. neei* red biotype (plants that gave rise to the BTH1 genotype).

Another response to salinity stress detected in both *S. neei* genotypes was the reduction of photosynthetic pigments, which can lead to inhibition of photosynthesis [19]. Costa et al. [13] found that chlorophyll a production in *S. neei* (sin. *Salicornia gaudichaudiana*) was more affected by salinity than by UV-B radiation levels. Tiku [35] also observed that chlorophyll a concentration in *Salicorina rubra* plants irrigated with 240 mM NaCl (14 NaCl g L^{-1}) culture solution was 72% smaller than in fresh water. In fact, succulence, lignification and changes in photosynthesis are adaptation mechanisms related to salt stress. Besides decreasing concentration of photosynthetic pigments, *S. neei* genotypes show chromatic adaptations against oxidative stress in photosystems induced by salt stress. BTH1 genotype seems to use its high carotenoid content to energy dissipation of the photosynthetic reaction centres (generate by no electrons fixation in CO_2 at the Calvin cycle) [20]. It was evidenced by the maintenance of high ratio of these photoprotective pigments against light-harvesting chlorophylls and the enhancement of de-epoxidation state (DES) of xanthophylls (Figure 1C) at increasing salt concentration. Under strong salt stress, violaxanthin is de-epoxidized and transformed into zeaxanthin, which plays a direct antioxidant role, acting as lipid protective [20,21]. Previously, Costa et al. [13] observed the elevation of zeaxanthin content in *Salicornia bigelovii* shoots exposed to increasing UV-B solar radiation and associated it with the protective effect of this compound against photooxidative damage of chloroplasts. According to Duarte et al. [18], the conversion of violaxanthin to zeaxanthin is one of the most effective energy dissipation mechanisms. Contrasting to their statement, BTH2 genotype mitigated photooxidative stress controlling the chlorophyll *a*:*b* ratio, which decreased due to the increment of chlorophyll b percentage in the total chlorophyll content along the salinity gradient (Figure 1B). Chlorophyll b has a protective function for photosystems due to the lower photo-oxidation rate compared to chlorophyll a [19].

Overall, the BTH2 *S. neei* genotype was more growth efficient and salt tolerant than the BTH1 genotype. The mechanism(s) for higher salt tolerance of BTH2 is (are) not clear and may be associated with metabolic pathways not evaluated in the present study. Duarte et al. [18] and Redondo-Gómez et al. [17] found strong evidences that *Salicornia fruticosa,* under salt stress, increases photorespiration and/or uses cyclic electron transport as an additional photo-protective mechanism. For instance, this later mechanism leads to an excessive accumulation of energy at the level of cyclic photophosphorylation in Photosystem I, where neither O_2 nor NADPH are produced. *Salicornia fruticosa* is a perennial Salicornioideae species closely related to *S. neei* and further studies are necessary to verify if the above-cited biochemical responses to salinity are also used by Brazilian sea asparagus genotypes.

4.2. Responses of S. neei Genotypes under Saline Effluent Irrigation at Field Conditions

The two new *S. neei* genotypes showed better growth performance and more succulent shoots in the field than during greenhouse experiment. Similar contrasting results were found with accessions of *Salicornia bigelovii* Torr. [7]. Different performances of *S. neei* genotypes between experiments can be associated with availability of solar irradiation for plants. Inside the greenhouse, the average solar irradiation was 6.86 MJ m^{-2} day^{-1}, less than half of the available light in the field (19.8 MJ m^{-2} day^{-1}). Field environmental conditions (approximately 230 mM salinity plus high solar irradiation) favoured the performance of BTH2 genotype, which showed higher values of growth parameters than BTH1. On the opposite, plants of the greenhouse experiment had similar height, shoot biomass, and BTH1 shoots had more branches than BTH2 shoots. These results corroborate the hypothesis of BTH2 *S. neei* genotype is more growth-efficient and stress-tolerant than BTH1.

Shoot heights reached by *S. neei* genotypes were higher than those obtained by Doncato and Costa [5] at previous field cultivation in the same location and of other sea asparagus species, for instance, *Salicornia persica* and *Salicornia fructicosa* [6]. Shoot biomass production of BTH2 *S. neei* (5.74 ton fw h^{-1}) irrigated with saline shrimp farm effluent was equivalent to values reached by

vegetables irrigated with fresh water, such as common asparagus (5.83 ton fw ha^{-1}). However, shoot biomass production of *S. neei* was lower than those of artichoke (12 ton fw ha^{-1}), spinach (14.1 ton fw ha^{-1}), and brassicas (e.g., broccoli and cauliflower; 16.4 ton fw ha^{-1}) [36]. It is also important to highlight that other field trials showed that *S. neei* can achieve shoot yield of 23 ton fw ha^{-1} under irrigation with saline shrimp farm effluent after 3–4 months of cultivation [9].

Additionally, *S. neei* shoots showed different phenolic acid profile and higher quercetin contents than plants cultivated in the greenhouse. Distinct environmental characteristics of these two cultivations should affect the tissue contents of phenolic compounds [13,18]. For instance, the greenhouse was covered with a 150 μm thick photostabilized LDPE Film with UV Absorber, and full UV exposure of *S. neei* in the field plot can explain the production of some phenolic compounds by sea asparagus plants [13]. Field cultivated BTH2 plants had an average FPC content higher than that of ethanolic and methanolic extracts of the vegetative shoots of green (respectively, 1.89 and 1.94 mg GAE g^{-1} dw; calculated considering 80% shoot water content) and red (respectively, 2.20 and 2.35 mg GAE g^{-1} dw) wild biotypes of *S. neei* from southern Brazil, cultivated with saline effluent of shrimp farm [12]. Free phenolic compounds and FFC of BTH2 shoots were higher than those found in methanolic extracts of wild plants of *Sarcocornia perennis* from Tunisia [37] and *Salicornia europaea* collected in Tukey [38], which range between 4.32–9.89 mg GAE g^{-1}dw and 3.3–5.1 mg catechin equivalent (ce) g^{-1} dw, respectively. The content of phenolic compounds in BTH2 shoots was also equal or greater than that of organic asparagus (FPC = 19.4 mg GAE g^{-1} dw; FFC = 5.28 mg ce g^{-1} dw) [39], artichoke (FPC = 0.84 mg GAE g^{-1} dw; FFC = 0.65 mg ce g^{-1} dw), spinach (FPC = 0.6 mg GAE g^{-1} dw; FFC = 0.03 mg ce g^{-1} dw) [40], broccoli and cauliflower (FPC ranged from 8.24 to 17.2 mg GAE g^{-1} dw) [41]. Quercetin content of BTH2 shoots was higher than that observed in artichoke and spinach (1.7–1.9 μg g^{-1} dw). Field cultivated BTH2 *S. neei* genotype demonstrated antioxidant capacity against DPPH radicals (IC_{50} = 5.41 mg dw mL^{-1}) higher than that of other sea asparagus (*Sarcocornia perennis alpine*—IC_{50} = 11.5 mg dw mL^{-1}; *Sarcocornia perennis perennis*—IC_{50} = 8.04 mg dw mL^{-1}) [10] but similar *Salicornia ramosissima* (IC_{50} = 5.69 mg dw mL^{-1}) [10], white cabbage (5.78 mg dw mL^{-1}) and turnip roots (6.92 mg dw mL^{-1}) [42].

The differences in antioxidant capacity between *S. neei* genotypes, as observed in field cultivated plants, can be associated with the distinct composition and individual quantities of phenolic compounds produced by each genotype. High contents of carotenoids, syringic acid and vanillin play an important role against oxidative stress for BHT1 genotype, as demonstrated by modulation of these compounds under increasing salinities, whereas high production of flavonoids (FFC and quercetin), and protocatechuic and caffeic acids by BHT2 plants in the field plot seems to be determinant of a 2.6-fold higher antioxidant activity of their shoot extracts than those from BTH1 plants. Indeed, the FFC content of BTH2 shoot represents around 25% of all phenolic compounds (FPC), a much higher percentage than BTH1 genotype (around 4% of FPC). Previously, Oh et al. [11] found quercetin as the most abundant phenolic compound detected in ethanolic extracts of *Salicornia herbacea* shoots, as well as isolated quercetin and catechuic acid were especially potent to scavenge DPPH radical. Phenylpropanoids, such as protocatechuic and caffeic acids that appear only in plants of *S. neei* cultivated in the field, are frequently concentrated in cuticles and cell walls exerting specific roles such as protection against oxidative damage, stabilizing reactive phenoxy radicals formed by intense UV radiation exposure [43].

5. Conclusions

The present study revealed that BTH1 and BTH2 genotypes of *S. neei* have high tolerances to salt stress but rely on distinct knowing photo-protective mechanisms and phenolic metabolites with antioxidant capacities. BTH1 genotype maintains high ratio of photoprotective carotenoids in relation to light-harvesting chlorophylls and enhances the conversion of violaxanthin to zeaxanthin in order to dissipate exceeding energy not used in the CO_2 assimilation process at increasing salt concentration. BTH2 genotype mitigates photooxidative stress increasing the relative content of chlorophyll b in the

photosynthetic pigments and by raising its shoot content of flavonoids (e.g., quercetin) to enhance antioxidant action. BTH2 genotype had higher growth, metabolites production and antioxidant capacity in the field experiment irrigated with shrimp farm effluent compared to BTH1 genotype and traditional crops irrigated with fresh water. Yield and contents of FPC and FFC in shoots of *S. neei* genotypes can be enhanced by cultivating plants under 34–86 mM NaCl. A further rise in salt concentration can induce the accumulation in shoot tissue of some bioactive metabolites, such as zeaxanthin and quercetin. Thus, salt stress might be applied before harvesting to increase these specific compounds in sea asparagus genotypes. Further studies must be done to find the optimal time when the increase of metabolite concentrations is still higher than the drawback of reduced growth through salinity.

Author Contributions: This work was a product of the combined effort of all authors. All authors conceptualized the study. C.S.B.C. conceived and designed the experiments and critically reviewed the manuscript; M.M.d.S. and K.B.D. performed the experiments, collected and analyzed the data, and drafted the manuscript; C.R.M. analyzed the data and drafted the manuscript; E.B.-F. and M.M.d.S. critically reviewed the manuscript.

Acknowledgments: This work was supported by the Brazilian National Research Council—CNPq (C.C.; grant number 573884/2008-0-INCTSAL). The authors were sponsored by Coordination for the Improvement of Higher Education Personnel—CAPES (M.M.d.S. and K.B.D.).

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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