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Influence of saline irrigation schedules on vegetative growth and reproduction of different progenies of the sea asparagus *Salicornia neei* Lag.

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Resumo

Influência de esquemas de irrigação salina no crescimento vegetativo e reprodução de diferentes progênies de aspargos marinhos *Salicornia neei* Lag. A *Salicornia neei* é utilizada como vegetal fresco em cozinhas gourmet e alimentos industrializados, bem como na alimentação animal. Este trabalho teve como objetivo avaliar o efeito de diferentes regimes de irrigação de água salina sobre o crescimento vegetativo de progênies F3 e F4 de novas linhagens de *S. neei* (BTH1 e BTH2). Vinte plantas por progênie foram irrigadas com 375 L de água salina da carcinicultura a cada dois (T2) e quatro (T4) dias em canteiros. Esses esquemas de irrigação foram quantificados como 249% e 151% do potencial de evapotranspiração estimada do período de estudo (670 mm), respectivamente. Os dados foram analisados por ANOVA. BTH2-F4 exibiu o melhor desempenho vegetativo e uma biomassa média da parte aérea fresca de 201,2 ± 17,1 g. O tempo médio para o início da floração foi mais cedo e o investimento em estruturas reprodutivas foi maior (representando 54-62% da biomassa total da parte aérea) para as progênies BTH1 do que BTH2 (14,6-16,7 semanas; 26-42% da biomassa total da parte aérea). A irrigação a cada quatro dias beneficiou a altura dos caules e atrasou o florescimento de ambas as linhagens, mas não afetou a produção de biomassa da parte aérea. Em conclusão, as plantas BTH2-F4 apresentaram melhor desenvolvimento entre as progênies e maior altura do caule sob irrigação a cada quatro dias.

Palavras-chave: Efluente de carcinicultura; Halófita; Sistema agro-aquacultura; Sistema BFT

Abstract

Salicornia neei is used as a fresh vegetable in gourmet kitchens, in industrialized food, and for animal food. This work evaluated the effect of different irrigation schedules of saline water on the vegetative growth of F3 and F4 progenies of new *S. neei* lineages (BTH1 and BTH2). Twenty plants per progeny were submitted to irrigation with 375 L of saline water from shrimp farming every two (T2) and four (T4) days in field plots. These irrigation schedules were quantified as 249% and 151% of the estimated potential evapotranspiration of the study period (670 mm), respectively. Data were analyzed by ANOVA. BTH2-F4 exhibited the best vegetative performance and an average fresh shoot biomass of 201.2 ± 17.1 g. The average time for onset of flowering was earlier and investment in reproductive structures was larger (representing 54–62% of the total shoot biomass) for the BTH1 progenies than BTH2 (14.6–16.7 weeks; 26–42% of the total shoot biomass). Irrigating every four days benefited shoot height and delayed flowering of both lineages but did not affect shoot biomass production. In conclusion, BTH2-F4 plants developed the best among the progenies and had tall shoots when irrigated every four days.

Key words: Agri-aquaculture system; BFT system; Halophyte; Shrimp farm effluent

Introduction

Salicornia neei Lag is a halophyte native to Brazilian salt marshes and mangroves (syn. *Sarcocornia ambigua* (Michx.) M.A. Alonso & M.B. Crespo, *Salicornia gaudichaudiana* Moq.; COSTA et al., 2019). This plant is sold as "sea asparagus" or "samphire" and used as a fresh vegetable in gourmet kitchens, in the industrial sector (e.g., pickles, salami, beer and biosalt), and for animal food (COSTA; HERRERA, 2016; ALVES et al., 2019). *Salicornia neei* shoots have high mineral content (DONCATO; COSTA, 2018a; 2018b; ALVES et al., 2020) and bioactive properties (COSTA et al., 2014; SOUZA et al., 2018). Nowadays, sea asparagus is a highly valued alternative vegetable in the European and North American markets (VENTURA et al., 2011; GLENN et al., 2013; VENTURA; SAGI, 2013). Field trials in coastal sandy soils (COSTA et al., 2014) and inland semiarid regions (COSTA; HERRERA, 2016; ALVES et al., 2019; 2020) showed that *S. neei* can achieve a shoot yield up to 23 t.ha^{-1} (fresh weight) after 3–7 months of cultivation under irrigation with saline water or shrimp farm effluent.

Shoot height and branch length are characteristics of agroeconomic interest for both fresh sea asparagus commercialization and industrialization of plant material. These morphological aspects can be maximized by selecting progenies with the best vegetative performance (i.e., shoot height), and breeding programs have been carried out with the commercial species *Salicornia bigelovii* (ZERAI et al., 2010). Since 2010, a breeding program of *S. neei* based on pure line selection from ecomorphotypes found in estuarine habitats has been carried out by the Laboratório de Biotecnologia de Halófitas (Instituto de Oceanografia, Universidade Federal do Rio Grande – FURG, Rio Grande, RS, Brazil) (DONCATO; COSTA, 2018a). Two lineages (BTH1 and BTH2) with contrasting prostrate and decumbent plants with green and reddish colorations, respectively, were obtained. Recent studies confirmed that these lineages show distinct genetic (COSTA et al., 2019), mineral (DONCATO; COSTA, 2018a; 2018b) and phenolic compositions (SOUZA et al., 2018). However, plant field trials are needed to evaluate how cultivation conditions affect growth and desirable agronomic traits of these lineages.

Regarding the cultivation conditions for halophytes, saline irrigation should be carried out with a periodicity capable of keeping the salt level in the soil within physiological limits that guarantee high productivity for the plants. Frequently, the main strategy to get the desirable salt concentration in the root zone is to apply irrigation water in excess of the potential evaporation rate. Field trials that had high yields of *Salicornia* species in semiarid regions were obtained by irrigating the soil with seawater (daily or twice daily) at water volumes surpassing 180–240% of the potential evaporation (GLENN et al., 2013; COSTA; HERRERA, 2016).

This study compared the vegetative growth, flowering and biomass production of F3 and F4 progenies of the lineages BTH1 and BTH2 of *S. neei* plants in a sandy soil when produced under different irrigation schedules with saline effluent from shrimp farming.

Material and Methods

This study used a total of four genotypes belonging to F3 and F4 progenies of the lineages BTH1 and BTH2 of *S. neei*. All the plants were obtained from seeds of the germplasm of the Laboratório de Biotecnologia de Halófitas (Federal University of Rio Grande – FURG, Rio Grande, RS, Brazil). After germination, seedlings were maintained for a total of 14 weeks in an unheated greenhouse before being transplanted at the cultivation site.

From November 2014 to April 2015, plants of *S. neei* were grown in field plots located at the Marine Station of Aquaculture (EMA/FURG) in Rio Grande, RS, Brazil (32°12'19"S; 52°10'45"W). According to Köppen's classification, the climate of the region is Cfb-mesothermal, warm-temperate, with a mean maximum temperature in the summer of approximately 27°C and a mean minimum temperature during the winter of approximately 10°C (RIBEIRO; COSTA, 2015). The annual averages for rainfall and potential evapotranspiration are 1,307 mm and 1,304 mm, respectively, with a dry period in the austral summer (Brazilian National Institute of Meteorology – INMET, Climatological Normal 1981-2010, http://www.inmet.gov.br).

Plants were grown in two plots (6.5 × 3.5 m), and both plots had eight planting rows that were 3.5 m long and separated by parallel grooves. Each plot received a different irrigation schedule and was divided into small subplots. Each subplot included two adjacent rows, with 10 cultivated plants per row, spaced 25 cm apart and 60 cm between planting rows. The four progenies were randomly assigned to the subplots, and each plant represented a replicate of the progeny treatment (n = 20 plants per progeny). Thus, the experimental design was in completely randomized blocks (F3 and F4 progenies of the lineages BTH1 and BTH2) with no replication for the factor irrigation schedule.

The irrigation schedule treatment throughout the trial consisted of two levels, with 375 L per plot of saline effluent from a tank of *Litopenaeus vannamei* shrimp cultivated in a Biofloc Technology (BFT) system, which corresponded to every 2 days (T2) and every 4 days (T4). Plants were watered by filling up drainage ditches and surface irrigation corresponded to 189% (T2) and 94% (T4) of the monthly potential evapotranspiration (ETo; calculated by the Penman-Monteith method) of the study period (135 mm per month), which was estimated based on hydric monitoring of the Decision Support System in Farming made available by the Brazilian Institute of Meteorology – INMET (http://sisdagro.inmet.gov.br/sisdagro/app/index). When rainfall was considered (see Results), T2 and T4 irrigation schedules were quantified as 249% and 151% of the estimated potential evapotranspiration of the study period (670 mm), respectively.

The soil of the experimental site was an Orthic Quartzarenic Neosol (i.e., new soil, undeveloped with a high content of sand that was well-drained and characteristic of the coastal plain of Rio Grande do Sul). The plot soil was separated from the surrounding soil by a geomembrane that was inserted in the soil at a depth of 1 m to form a barrier. The saline effluent was the main source of nutrients and water for the *S. neei* plants. During the growth period, the average values (± standard error; n = 23) of effluent salinity, pH, nitrate, ammonium and phosphate were 12.45 ± 0.21 g NaCl L^{-1} (≈18.50 dS m^{-1}), 8.65 ± 0.04, 4.90 ± 0.66 mg NO_3-N L^{-1}, 0.15 ± 0.13 mg NH_4-N L^{-1} and 0.30 ± 0.05 mg PO_4-P L^{-1}, respectively.

In both plots, soil samples were collected twice a week from planting rows 1, 4 and 8 at depths of 0–10 cm. The soil moisture content was determined by the gravimetric method, and the soil electrical conductivity was estimated from 1:2 dry soil-distilled water ratio extracts ($CE_{1:2}$) using a Hanna HI9835 conductivity meter. The daily data for precipitation, air temperature (minimum and maximum) and radiation were obtained from the INMET station located on the FURG campus (32°04'43"S; 52°10'03"W), approximately 20 km from the field site. The differences between soil moisture content and $CE_{1:2}$ among the two irrigation schedules (T2 and T4), three sampling points of the plots and two climatic seasons of the cultivation period (spring – early summer and mid-summer – autumn) were tested with a three-way ANOVA (ZAR, 2010). The introduction of the climatic season factor was due to the marked seasonal variation of rainfall (see Results).

Shoot biometry was conducted at planting (14-week-old seedlings) and after 22 weeks of plot cultivation. The height (cm), number of primary branches of the shoots and length of the longest branch (cm) were quantified. The differences between the initial and final values of height and number of branches of each plant were used to calculate the absolute vertical growth rate (AVG; cm per week) and absolute rate of branch formation (ABF; branches per week), respectively. The plants were monitored weekly for the presence of reproductive structures (stigma, anther, and reproductive segments with seeds). These data were used to calculate the average pre-reproductive length period (PRL; time spent until the beginning of flowering) and percentage of flowering plants for each progeny (ZERAI et al., 2010; ALVES et al., 2019).

At the end of cultivation, all the plants (mostly mature) were harvested just above ground level. In the laboratory, the plant samples were washed to remove any soil and vegetative shoot and reproductive shoot segments (with flowers or seeds) were separated and weighed on a precision scale to determine the fresh weight. Subsequently, all the samples were oven dried at 60°C for 48 h and weighed on the precision scale to determine the dry weight.

Biometric measurement, reproductive and biomass data were compared among four levels of the progeny treatment (F3 and F4 progenies of the lineages BTH1 and BTH2) and two levels of the irrigation schedule treatment (T2 and T4) by two-way nested analyses of variance (ANOVAs) with irrigation schedule as the fixed factor and progeny as the random factor nested in the irrigation schedule. The ANOVAs were followed with a comparison by Tukey's HSD test at 5% significance. In order to meet the prerequisites of normality and homoscedasticity (ZAR, 2010), the initial height and longest branch, final height and fresh weights of vegetative segments were transformed by $\log_{10}(x)$. Final longest branch and total shoot biomass were transformed by square root, and AVG was transformed by $\log_{10}(x+1)$. Differences among progenies in the percentage of flowering plants were tested by a Chi-square test (χ^2) at 5% significance (ZAR, 2010).

Results

During cultivation, the average minimum and maximum air temperatures were 22.8 and 24.0ºC, respectively. The average (± standard error) for solar radiation was 1,835.5 ± 25.9 kJ.m^{-2} per hour. The daily precipitation ranged between 0.0 and 7.5 mm per day, and 340 mm accumulated by the end of 22 weeks of cultivation. Precipitation showed a marked seasonal variation, and it rained only 20 mm between February and April 2015 (mid-summer – autumn period). This rainfall pattern significantly ($p < 0.001$) affected both soil moisture (Table 1) and CE$_{1:2}$ of the experimental plots (Table 1). For instance, the CE$_{1:2}$ showed average values significantly lower in the spring – early summer period (7.3-8.8 dS.m^{-1}) than in the mid-summer – autumn period (15.1-15.6 dS.m^{-1}). The irrigation schedule significantly ($p < 0.05$) affected soil moisture (Table 1), which had global averages of 13.8 ± 0.6% for T2 and 12.3 ± 0.7% for T4.

The *S. neei* BTH2-F4 progeny had the best performing plants (Table 2), which were 20–40% taller, 13–15% more branched and had branches 30–60% longer than the other progenies (Figure 1A-C). Height growth rates, estimated by differences in shoot height at the beginning and end of the experiment, ranged from 0.9–1.2 cm.week^{-1} and 1.0–1.3 cm.week^{-1} for the irrigation schedules T2 and T4, respectively. BTH2 plants already had a higher average number of branches on their shoots at the planting date (24.9 branches) than the BTH1 plants (17.9 branches), averaging 50–60 branches after 22 weeks of cultivation (Figure 1B) or the formation of 1.2–1.7 branches per week.

The average time for onset of flowering was different ($p < 0.001$) among progenies (Table 3). Flowering was earlier for the BTH1 progenies (12.6–14.0 weeks) compared to BTH2 (14.6–16.7 weeks). The final percentage of flowering plants from the four *S. neei* progenies ranged from 80 to 100%, with no significant differences among progenies for T2 ($\chi^2 = 0.62$; $p = 0.89$), T4 ($\chi^2 = 0.59$; $p = 0.90$), and the combined data from the two treatment plots ($\chi^2 = 1.22$; $p = 0.99$).

Since the statistical results for the fresh and dry weights of the different components of shoot biomass were similar, only fresh weight data are presented below (Table 3). There were differences among progenies regarding fresh biomass of BTH2-F4 shoots (201.2 ± 17.1 g) that were, on average, 41.1–93.4% heavier than those of BTH1 progenies (F3 = 104.0 ± 8.9 g and F4 = 142.6 ± 12.1g) (Figure 1D).

TABLE 1: Average (± standard error) and three-way ANOVA for soil moisture and soil electrical conductivity ($CE_{1:2}$) of *Salicornia neei* field plots subjected to irrigation with saline effluent from shrimp farming every two (T2) and four (T4) days. Sampling points inside the plots and two climatic seasons of the year (SES = spring – early summer; MSA = mid-summer – autumn) were also considered in ANOVA models.

	Moisture (%)		**Electrical Conductivity ($dS.m^{-1}$)**	
	SES	MSA	SES	MSA
T2	17.94	9.72	8.83	15.56
	(0.79)	(0.40)	(1.62)	(0.83)
T4	16.71	7.90	7.34	15.07
	(0.92)	(0.47)	(0.96)	(0.96)
	F	P	F	p
Irrigation treatment (I)	4.11	*	0.54	ns
Sampling point (P)	0.38	ns	0.19	ns
Season (S)	125.99	***	19.37	***
I x P	0.27	ns	1.09	ns
I x S	0.15	ns	0.07	ns
P x S	0.05	ns	0.44	ns
I X P X S	0.01	ns	0.08	ns

* $p < 0.05$; *** $p < 0.001$; ns: non-significant.

TABLE 2: Results of two-way analysis of variances (ANOVA) of shoot biometry, total shoot biomass and absolute rates of four progenies of *Salicornia neei* (f3 and f4 progenies of the lineages BTH1 and BTH2) subject to irrigation with saline effluent of shrimp farming every two (T2) and four (T4) days.

Variável	**F(Progeny)**	**p**	**F(Irrigation)**	**p**
Shoot height (cm)	10.82	***	4.88	*
Branch number	4.41	***	1.66	ns
Longest branch length (cm)	8.23	***	1.30	ns
AVG (cm per week)	2.54	*	2.18	ns
ABF (cm per week)	3.35	**	0.57	ns
Total shoot biomass (g)	5.57	***	2.93	ns

* $p < 0.05$; *** $p < 0.001$; ns: non-significant.

FIGURE 1: Average (± standard error) of shoot height (A), branch number (B), longest branch length (C), total shoot biomass (D) of four progenies of *Salicornia neei* after 22 weeks of irrigation with saline effluent of shrimp farming every two (T2) and four (T4) days. Different lowercase letters represent significant differences between the averages ($p < 0.05$), according to the Tukey's HSD test.

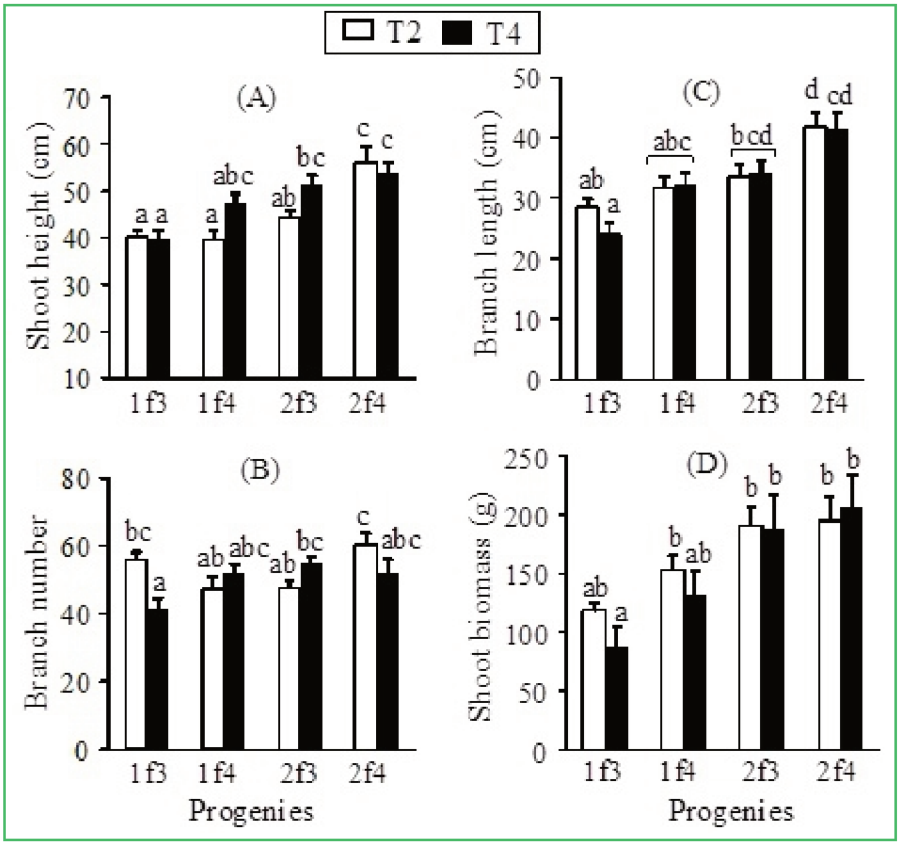

TABLE 3: Average (± standard error) and two-way ANOVA for vegetative andvegetative and reproductive shoot biomass, reproductive biomass allocation, flowering time and percentage of flowering shoots of the four progenies of *Salicornia neei* subject to irrigation with saline effluent of shrimp farming every two (T2) and four (T4) days.

Irrigation / Progeny	Vegetative biomass (g)		Reproductive biomass (g)	Reproductive allocation (%)		Flowering time (weeks)		Flowering (%)
T2								
BTH1-f3	56.39	ab	95.95	62.14	bc	12.69	a	100
	(4.54)		(11.01)	(3.95)		(0.13)		
BTH1-f4	91.72	abc	105.60	53.37	abc	12.63	a	100
	(16.50)		(22.08)	(6.13)		(0.42)		
BTH2-f3	158.29	bc	115.23	41.90	abc	15.86	cd	100
	(13.93)		(15.93)	(4.27)		(0.31)		
BTH2-f4	167.10	bc	90.93	35.32	ab	14.57	bc	80
	(44.17)		(23.14)	(1.87)		(0.37)		
T4								

BTH1-f3	50.81	a	112.02		68.73	c	14.03	b	100
	(7.31)		(17.06)		(0.75)		(0.26)		
BTH1-f4	132.50	abc	78.22		46.01	abc	12.63	a	100
	(68.55)		(24.76)		(14.73)		(0.18)		
BTH2-f3	184.32	c	120.78		38.60	ab	16.70	d	89
	(41.86)		(30.97)		(4.76)		(0.60)		
BTH2-f4	264.99	c	89.87		26.65	a	15.46	bcd	80
	(52.29)		(12.46)		(2.97)		(0.22)		
F_p	7.02	***	0.59	ns	5.63	***	28.70	***	
F_I	0.53	ns	0.01	ns	0.50	ns	11.90	***	

* $p < 0.05$; *** $p < 0.001$; ns: non-significant. Different lowercase letters (within a column) represent significant differences between the averages ($p < 0.05$), according to the Tukey's HSD test.

Shoot reproductive biomass did not differ among progenies (P) and irrigation treatments (I) (Table 3). However, lineages showed a contrasting result in their reproductive allocation. BTH1 progenies invested significantly more (Tukey test $p < 0.05$) in reproductive structures than progenies of the BTH2 lineage. BTH1-F3 allocated between 54–62% of the total shoot biomass to reproductive segments, which represents 63–111% more than the BTH2 progenies invested in reproductive structures (Table 3).

The extension of the irrigation schedule had a small effect on *S. neei* growth, with T4 plants having significantly (6.6%) taller shoots (global average = 48.1 ± 1.2 cm) than those of T2 (45.1 ± 1.3 cm) (Table 2). Additionally, T2 plants had a significantly shorter pre-reproductive period than T4 plants (on average about one week) (Table 3). BTH1-F3 had a reduced number of shoot branches for T4 (Table 2).

After the cultivation period, the global average of total fresh shoot biomass of *S. neei* plants was 164.7 ± 8.3 g for T2 and 154.3 ± 12.9 g for T4, but no significant differences were found for the irrigation schedule treatment (Table 2).

Discussion

There were significant differences in plant growth among *S. neei* progenies. Taller branching shoots with longer branches of BTH2-F4 in relation to other *S. neei* progenies were also observed by Doncato and Costa (2018a) for a crop irrigated with shrimp farming water. Shoot height and branching are characteristic of genetic differences between *Salicornia* populations (ZERAI et al., 2010; MILIĆ et al., 2011; VENTURA et al., 2011). The growth in height of the BTH2-F4 shoots (Figure 1A) was similar to that described for *Sarcocornia fruticosa* "ecotypes" grown in saline solutions with 25–100% seawater content (VENTURA et al., 2011), which after 9 weeks of cultivation reached the commercial height of 10 cm used for sea asparagus in Israel (VENTURA et al. 2011; VENTURA; SAGI, 2013). The rates of branch formation for *S. neei* shoots were similar to those observed for *Sarcocornia perennis* after 6 weeks of growth in unsalted saturated soil (1.9 branches per week) and salinized soil at 52 dS m^{-1} (35 g NaCl L^{-1}; 1.1 branches per week) (ADAMS; BATE, 1994).

The length of the pre-reproductive period of *S. neei* progenies (12.6 to 16.7 weeks after planting) was half of that observed for native plants of this species in southern Brazil, which germinate in salt marshes in August and start flowering in February (after 25 weeks) of the following year (AZEVEDO, 2000). However, the time to onset of flowering was similar to *S. neei* plants irrigated with saline water in northeastern Brazil (12–16 weeks, ALVES et al., 2019) and to commercial plants of *Salicornia bigelovii* in breeding programs in Eritrea (16.6 weeks, ZERAI et al., 2010). This distinct behavior of cultivated plants seems to be caused by using plants that were pre-grown for several weeks inside greenhouses before planting, whose advanced development stage makes it possible to shorten the pre-reproductive period. The later flowering and lower investment in reproductive structures of BTH2 plants seem to be a genetic differentiation that resulted from the selection of this lineage, where large shoot development of its ancestors was prioritized (DONCATO; COSTA, 2018a). On the other hand, the high reproductive investment of BTH1 is also a feature of agronomic interest, since *S. neei* reproductive shoot biomass (with seeds) can be a raw material for edible oil and biofuel production (D'OCA et al., 2012; COSTA et al., 2014).

The extension of the irrigation schedule apparently favors vegetative growth of *S. neei* plants. Similarly, Woo and Takekawa (2012) cultivated *Sarcocornia pacifica* with daily inundations of 25% (6 h), 50% (12 h), 75% (18 h) and 100% (24 h), at different salinities (0, 10, 20 and 30 g NaCl.L^{-1}), and found that plants inundated 25% of the time grew significantly taller than those inundated 75% or more of the time. The experimental plots showed similar seasonal variation in soil electrical conductivity but no statistical differences in their overall averages, which ranged (7.3–15.6 dS.m^{-1}) within the limits of moderate salinities where *S. neei* genotypes showed maximum growth (SOUZA et al., 2018). Costa and Herrera (2016) reviewed data of several crops of *S. neei* under saline irrigation and found that the best plant development occurred when the average soil moisture was close to 6–8% in both sandy and podzolic soils. Long-term maintenance of soil moisture near saturation (i.e., above 20% water content in the local fine sandy soil) seems to inhibit plant development, possibly due to the establishment of hypoxic conditions and/or accumulation of toxic compounds resulting from the metabolism of anaerobic microorganisms (WOO; TAKEKAWA, 2012; MIRLEAN; COSTA, 2017). *Salicornia neei* grows in the middle-upper tidal levels of salt marshes (AZEVEDO, 2000; DONCATO; COSTA, 2018a; COSTA et al., 2019), where plants are subjected to several hours of daily exposure and drained soils in their natural habitat. Lower soil moisture was probably the main environmental factor responsible for the overall tallest shoots under the T4 irrigation schedule, which maintains the plants closest to their optimum water status. In contrast, BTH1-F3 had a reduced number of shoot branches for T4 (Figure 1B).

The presence of a geomembrane in the field plots and the rainfall, concentrated mainly during the spring and early summer, retained moisture in the soil and reduced the effect of the different irrigation schedules. The T2 and T4 irrigation schedules were quantified as 249% and 151% of the estimated potential evapotranspiration of the study period (670 mm), and both values are well within the recommended range of irrigation water in excess of the potential evaporation rate that is needed for saltwater irrigation of halophytic species in order to avoid salt concentration in the root zone (GLENN et al., 2013; COSTA; HERRERA, 2016).

Among the studied progenies, BTH2-F4 had greater vegetative development. Its height increased with an irrigation schedule of every four days of saline effluent from a shrimp tank, which corresponds to 151% of the estimated potential evapotranspiration during the cultivation period.

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