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Saline irrigation schedules on mineral composition of sea asparagus *Salicornia neei* Lag. progenies

Regimes de rega salina na composição mineral de progénies de espargo do mar *Salicornia neei* Lag.

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A B S T R A C T

Sea asparagus (*Salicornia neei* Lag.) is a gourmet vegetable that has been experimentally studied due to its high nutritional quality. Saline irrigation may influence several chemical aspects of the plant. Therefore, the objective of this study is to investigate the mineral composition of *S. neei* progenies subjected to cultivation with two saline irrigation schedules from marine shrimp farming effluent. The cultivation was carried out with four progenies (two biotypes: BTH1 and BTH2; two generations: F_3 and F_4) of *S. neei* in field plots, each plot corresponded to an irrigation regime (T2: every two days; T4: every four days). The results of this study suggest that irrigation influenced plants' mineral composition. Few nutritional characteristics (e.g. N, Ca, Cu and Mn) of *S. neei* progenies were affected by the irrigation experiment, demonstrating the high degree of adaptation of this species to water/saline stress. The more prolonged regime of irrigation (i.e. T4) was the most suitable for plant production, as the plants presented a higher N content and lower Cu and Mn content than T2. For differences between progenies, the BTH2 lineage showed greater accumulation of P and Mg, which makes it an option as a gourmet vegetable in saline agriculture.

Keywords: agri-aquaculture system, BFT system, halophyte, shrimp farm effluent, nutritive potential.

R E S U M O

O espargo do mar (*Salicornia neei* Lag.) é um vegetal gourmet que vem sendo estudado experimentalmente devido à sua alta qualidade nutricional. A rega salina pode influenciar vários aspectos químicos da planta. Portanto, o objetivo deste estudo é investigar a composição mineral de progénies de *S. neei* submetidas ao cultivo com dois regimes de rega salina de efluente de criação de camarão marinho. O cultivo foi realizado com quatro progénies (dois biótipos: BTH1 e BTH2; duas gerações: F_3 e F_4) de *S. neei* em canteiros, cada canteiro correspondeu a um regime de rega (T2: a cada dois dias; T4: a cada quatro dias). Os resultados deste estudo sugerem que a rega influenciou a composição mineral das plantas. Poucas características nutricionais (e.g. N, Ca, Cu e Mn) das progénies de *S. neei* foram afetadas pelo experimento de rega, demonstrando o alto grau de adaptação desta espécie ao estresse hídrico/salino. O regime de rega mais prolongado (i.e. T4) foi o mais adequado para a produção de plantas, pois estas apresentaram um maior teor de N e menores teores de Cu e Mn do que T2. Para diferenças entre progénies, a linhagem BTH2 apresentou maior acumulação de P e Mg, o que a torna uma opção como vegetal gourmet na agricultura salina.

Palavras-chave: sistema agri-aquicultura, sistema de bioflocos, halófita, efluente da produção de camarão, potencial nutritivo.

INTRODUCTION

Sea asparagus (*Salicornia neei* Lag.) is a perennial species distributed in salt marshes and mangroves of the Atlantic and Pacific coasts of South America, as well as in the saline lowlands of central-south Argentina (Costa *et al*., 2019), characterized as a gourmet vegetable with high nutritional quality. Its shoots and seeds have a high mineral content (syn. *Sarcocornia ambigua* - Bertin *et al*., 2014; Doncato & Costa, 2018; Alves *et al*., 2020), as well as chemical characteristics (e.g. polyunsaturated fatty acids, such as linoleic acid and oleic acid), which can potentially be used for consumption (Costa, 2006; Bertin *et al*., 2014; Costa *et al*., 2014a), biofuel production and in the pharmaceutical industry (EPAGRI, 2008; Bertin *et al*., 2014; Costa *et al*., 2014a). Such aspects can be maximized from the selection of progenies with certain characteristics of agro-economic interest, as it has been done with other species of the genus Amaranthaceae, such as the commercial species *Salicornia bigelovii* (Zerai *et al*., 2010). In 2010, a selection program was started at the Laboratory of Biotechnology of Halophytes (BTH) linked to the Federal University of Rio Grande (FURG), through the identification and crossing of two biotypes of natural populations of *S. neei* from southern Brazil by pure lines, described in Doncato & Costa (2018).

This species is being experimentally cultivated in the field under irrigation with saline water (Costa *et al*., 2014b; Alves *et al*., 2020) and with aquaculture water or effluent (Costa, 2006; Costa *et al*., 2014a; Doncato & Costa, 2018, 2022). Recirculating waters and effluents of aquaculture are rich in nutrients that essential for plant nutrition. Previous studies have shown that Biofloc Technology (BFT) system of marine shrimp farming as water source for halophytes, like *S. neei*, can provide all the macronutrients and micronutrients required for mineral nutrition of plants without the need of supplementation (Doncato & Costa, 2021, 2023a, 2023b). To maximize the production and nutritional composition of plants, irrigation is one of the management practices that must be adapted to the growing condition. According to Lieth & Mansoom (1993), saline irrigation must be performed frequently to maintain a constant salinity level, adapting the irrigation frequency to the growing condition. For example, in sandy soils with low water retention rates and dry or seasonally dry climates, daily irrigation is required. Soil salinity level must be monitored to maintain yield and avoid reduced productivity (Glenn *et al*., 1999). Under surface irrigation with high saline water, halophytes often require a leaching percentage of 30% above their water use, so that excess salt accumulated near the roots during periods between irrigations is removed (Glenn *et al*., 1997, 2013).

The chemical composition of plants can also be markedly affected by saline conditions (Costa, 2006; Ventura *et al*., 2011; Duarte *et al*., 2014). For example, with increasing irrigation salinity, sodium accumulates in the plant vacuole while nitrogen osmolytes are produced and stored in the cytosol in order to stabilize protoplasmic structures (Glenn *et al*., 1999; Davy *et al*., 2006; Flowers *et al*., 2010; Duarte *et al*., 2014; Alves *et al*., 2020). According to Lieth & Mansoom (1993), the concentration of minerals in halophytes usually increases by 20-40% with the transfer of mixohaline waters to sea level salinity, with sodium being the largest accumulated cation. Ventura *et al*. (2011) observed that *Salicornia* irrigated with seawater diluted in different percentages, showed a noticeable increase in sodium and subtle potassium, in 100% of seawater. Furthermore, at this saline concentration, there was an increase in the percentage of total polyphenols (Bertin *et al*., 2014), which are recognized as antioxidant substances.

This increase in essential minerals and bioactive substances in plant tissue represents an improvement in the nutritional composition of plants under irrigation with high saline concentration. Several studies (Luque *et al*., 1999; Curado *et al*., 2014; Smillie, 2015) reported that *Salicornia* has great relevance in the bioaccumulation of metallic elements such as Al, Fe, Zn, Mn and Cu. A better understanding of the effects of saline irrigation on the nutritional qualities of *S. neei* progenies is needed. In our previous reports, we investigated the influence of saline irrigation on vegetative growth, flowering and biomass production of *S. neei* progenies (Doncato & Costa, 2018, 2022). The present study aimed to investigate variations in the mineral composition of *S. neei* progenies subjected to irrigated field cultivation with two saline irrigation schedules from marine shrimp farming (BFT system).

MATERIALS AND METHODS

The selection of *S. neei* progenies and the experimental design were described in detail in our previous studies, respectively (Doncato & Costa, 2018, 2022). In brief, from November 2014 to April 2015, F_3 and F_4 progenies of the lineages BTH1 and BTH2 of *S. neei* were grown in two field plots (6.5 × 3.5 m) with sandy soil, irrigated with two distinct schedules, with 375 L per plot of saline effluent from a tank of *Litopenaeus vannamei* shrimp cultivated in a BFT system. It is a minimal water exchange system where bacteria convert ammonia to nitrate, allowing a low-toxicity nitrogen compound to accumulate without reducing water quality (Doncato & Costa, 2023a). The irrigation schedule treatments were named T2 (irrigation every two days) and T4 (irrigation every four days), which corresponded to 189% (T2) and 94% (T4) of the monthly potential evapotranspiration (Eto; calculated by the Penman-Monteith method; Allen *et al*., 1998) of the study period (135 mm per month). In each plot, the four progenies were randomly assigned to the subplots, and each plant represented a replicate of the progeny treatment (n = 20 plants per progeny). The experimental design was in completely randomized blocks (F_3 and F_4 progenies of the lineages BTH1 and BTH2) with no replication for the factor irrigation schedule.

At the end of the experiment, five dry shoot samples (vegetative segments only) from each progeny were ground in a pestle mortar, subsequently subjected to nitric-perchloric and sulfuric digestion (for nitrogen analysis), according to the methodology described by Tedesco *et al*. (1995). With the extracts obtained in the digestions, nitrogen (N) was determined by distillation and titration (Tedesco *et al*., 1995), phosphorus (P) with the ultraviolet visible spectrophotometer, potassium (K) by flame photometry, as well as calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) were determined by atomic absorption spectrophotometer. Only essential plant mineral microelements important for human consumption were analyzed, since *S. neei* is cultivated for biomass aimed at food. These chemical analyses were performed by the soil laboratory of the Federal University of Pelotas (UFPel; Brazil).

Data was analyzed 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.

RESULTS

The different irrigation schedules significantly affected only the macroelements N (18% higher in T4) and Ca (16% higher in T2) (Table 1). Concerning microelements, only Mn and Cu were affected by irrigation schedules, with the contents of both elements being higher in T2. For instance, BTH2-F_3 plants showed 67% more Mn levels in the treatment T2 than in the treatment T4. The overall Cu average of plants from T2 were 54% higher than the one from T4 (Table 1). The BTH2 lineage had a significantly higher content of P (F_4) and Mg (F_3) than BTH1 (Table 1). These differences were 37.2% higher for P concentration than BTH1-F_4 and 17-18% higher for Mg content compared to the BTH1 lineage. Regarding to microelements, differences between progenies occurred for Fe and Cu only, but with the higher values occurring in BTH1 plants, which contained an average of 48% more Fe (F_3) and 39% more Cu (F_4) than BTH2-F_3 (Table 1).

DISCUSSION

Throughout the experiment, it became evident that *S. neei* was easily cultivated in the field plots with sandy soil under different irrigation schedules with saline shrimp effluent, which was the only source of nutrients available to the plants. This different irrigation schedules significantly influenced the mineral composition (e.g. N, Ca, Cu and Mn). Additionally, the lineages maintained most of the differences in terms of irrigation schedules and BTH2 had great mineral content, with emphasis on the F_4 progeny. This great mineral content under prolonged irrigation periods in also supported by a high vegetative performance (Doncato & Costa, 2022).

Table 1 - Average (± standard error) of the mineral composition (macronutrients presented in g Kg^{-1} and micronutrients presented in mg Kg^{-1}) of four progenies of *Salicornia neei* shoots in the irrigation treatments T2 (every two days) and T4 (every four days) and two-factor nested analysis of variance (ANOVA) of the composition of mineral elements

Progeny/ irrigation	N		P		K	Ca		Mg		Cu		Zn	Fe		Mn	
T2																
BTH1-F_3	10.22	a	2.13	ab	20.36	5.65	ab	12.26	a	10.39	bc	17.27	358.14	c	62.01	a
	(0.33)		(0.12)		(0.99)	(1.05)		(0.54)		(0.59)		(0.84)	(82.74)		(4.24)	
BTH1-F_4	11.40	ab	2.10	ab	18.73	5.31	ab	13.79	ab	13.13	c	16.60	265.22	abc	75.77	ab
	(0.73)		(0.22)		(1.32)	(0.48)		(1.03)		(1.69)		(1.84)	(51.57)		(7.54)	
BTH2-F_3	14.43	abc	2.34	ab	19.44	6.26	b	16.18	b	9.41	bc	19.91	244.03	abc	99.33	b
	(1.18)		(0.14)		(1.72)	(0.39)		(0.73)		(1.01)		(2.60)	(46.98)		(13.56)	
BTH2-F_4	14.22	abc	2.44	ab	18.15	6.58	b	13.69	ab	10.78	bc	24.10	230.50	abc	101.32	b
	(2.53)		(0.43)		(2.13)	(0.35)		(1.05)		(1.58)		(3.62)	(35.63)		(8.05)	
T4																
BTH1-F_3	16.14	bc	2.73	ab	20.20	5.31	ab	13.50	ab	8.43	abc	23.17	272.88	bc	72.67	ab
	(1.28)		(0.27)		(0.48)	(0.64)		(0.57)		(0.91)		(1.76)	(17.38)		(5.32)	
BTH1-F_4	13.08	abc	1.84	a	20.78	5.05	ab	11.56	a	7.25	ab	20.17	224.89	abc	72.30	ab
	(0.66)		(0.10)		(0.71)	(0.61)		(0.72)		(0.24)		(1.22)	(8.27)		(7.66)	
BTH2-F_3	13.36	abc	2.17	ab	17.78	4.60	a	13.84	ab	5.29	a	18.36	181.49	a	59.65	a
	(0.42)		(0.11)		(1.84)	(0.04)		(0.70)		(0.39)		(1.03)	(29.35)		(4.69)	
BTH2-F_4	16.84	c	2.97	b	22.34	5.52	ab	13.87	ab	7.45	ab	23.79	201.92	ab	71.68	ab
	(0.98)		(0.21)		(1.83)	(0.36)		(0.48)		(0.91)		(2.02)	(26.26)		(2.82)	
F p	2.64	ns	3.70	*	0.55	0.94	ns	3.97	*	3.76	*	2.89	3.12	*	2.40	ns
F i	10.58	**	1.27	ns	1.11	4.18	*	2.16	ns	33.21	***	1.72	2.65	ns	8.77	**
F pxi	3.44	*	2.27	ns	1.48	0.71	ns	2.78	ns	1.24	ns	1.41	0.13	ns	4.89	**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: non-significant ($p > 0.05$).
F p = progeny; F i = irrigation; F pxi = interaction between progeny and irrigation.
Different lowercase letters (within a column) represent significant differences between the averages ($p < 0.05$), according to the Tukey's HSD test.

Irrigation schedule effects

Few nutritional characteristics of *S. neei* progenies were affected by the irrigation experiment, demonstrating the high degree of adaptation of this species to water/salt stress. Most glycophyte species and even many halophytes when subjected to the same conditions of soil electrical conductivity and high NaCl contents would lead to an ionic imbalance (Flowers *et al*., 2010; Ventura *et al*., 2011; Rozema & Schat, 2013). Extended period between irrigations (i.e. T4) resulted in significantly lower concentrations of the minerals Ca, Cu and Mn in *S. neei* shoots, but particularly of BTH2-F_3. Additionally, N shoot content increased with prolonged irrigation.

The lower moisture and higher saline contents in the T4 field plot (e.g. summer-autumn; Doncato & Costa, 2022) may have led to the differences in N and Ca contents in the shoots between irrigation treatments. Under salt and hydric stresses, both annual (Davy *et al*., 2001) and perennial (Davy *et al*., 2006) *Salicornia* species produce and accumulate low molecular weight nitrogen compounds (markedly glycine-betaine), mainly in chloroplasts (Duarte *et al*., 2014), allowing an intracellular osmotic balance with the vacuole, where a large amount of NaCl is compartmentalized (Glenn *et al*., 1999; Zheng *et al*., 2009; Ventura *et al*., 2011; Duarte *et al*., 2014; Alves *et al*., 2020). *Salicornia neei* also accumulated soluble amino-acids in their shoots under water stress conditions (Alves *et al*., 2020). Consequently, the increase in N content in *S. neei* shoots from the field plot subjected to greater water stress (i.e. T4) was expected. On the other hand, Zheng *et al*. (2009) highlighted that the levels of Ca and K in *Salicornia europaea* shoots decreased with the increasing of salinity in the cultivation. These elements were maintained in plant roots, leading these authors to conclude that Ca and K are strongly involved in root cell ionic homeostasis. In a previous study (Doncato & Costa, 2018), BTH2 plants maintained high levels of Ca in their roots, suggesting a translocation mechanism of Ca into roots under more saline soil, and reduction of this element in *S. neei* shoots may also occur.

As for the Cu and Mn shoot contents, lower values in T4 plot may be resulted from lower input (via saline effluent loading) and lesser accumulation of these elements than in T2 plot. Smillie (2015) showed that the Cu content in *Salicornia* spp. shoots is directly related to its content in the sediment. Additionally, these same authors point out that, during the period of rapid pre-fruiting vegetative growth, less essential or non-essential metals, such as Cu, Fe and Mn, could be diluted by tissue expansion. Due to the higher growth of *S. neei* in T4 (Doncato & Costa, 2022), this dilution process may have been more marked, leading to differences between irrigation treatments.

Comparison of the mineral content

BTH2 lineage showed significantly higher levels of P and Mg than BTH1 lineage, while the microelements Fe and Cu remained more concentrated in the shoots of BTH1 lineage. Except for P, previously found in higher content in BTH1 lineage than in BTH2 lineage, these results were similar those described by Doncato & Costa (2018). Shoot contents of P and Mg in the plants that showed the best performance were higher than that observed in *S. neei* from salt marshes (syn. *Sarcocornia perennis* - Medina *et al*., 2008; Bertin *et al*., 2014) and *S. bigelovii* (Lu *et al*., 2010). The content of Mg in *S. neei* shoots can be 2-6 times higher than in gourmet vegetables such as *Asparagus officinalis* (2.0 g Kg^{-1} DW; Makus, 1994) and *Spinacea oleracea* (4.3 g Kg^{-1} DW; Sheikhi & Ronaghi, 2012).

In general, high but not toxic content of microelements were found in shoots of all *S. neei* progenies cultivated with saline effluent. Tissue content of these elements in annual and perennial species of *Salicornia* are directly dependent on soil/irrigation water concentrations, and their species are important bioindicators or bioaccumulators of metals (Luque *et al*., 1999; Curado *et al*., 2014; Smillie, 2015). In this way, great variations in the levels of metals are cited in the literature and toxic levels of these elements can be observed in plants of some localities.

The average Fe contents in *S. neei* shoots were 102-304% higher than those mentioned for *S. perennis*, *Salicornia stricta* (50-90 mg Kg^{-1} DW; Gorham & Gorham, 1955), *S. bigelovii* (86.4 mg Kg^{-1} DW; Lu *et al*., 2010) and *A. officinalis* (99.9 mg Kg^{-1} DW; Makus, 1994). Average of Mn concentration in BTH2-F_4 (71.68 mg Kg^{-1} DW) was higher than in *S. stricta* shoots from eastern England (60 mg Kg^{-1} DW; Gorham & Gorham, 1955), *S. perennis* (20-81.2 mg Kg^{-1} DW; Gorham & Gorham, 1955; Luque *et al*., 1999) and the vegetable *A. officinalis* (21.4 mg Kg^{-1} DW; Makus, 1994). The contents of Cu and Zn were lower than those mentioned for cultivated plants of *S. bigelovii* (7.9 and 35.0 mg Kg^{-1} DW, respectively; Lu *et al*., 2010), *A. officinalis* (18 and 77.3 mg Kg^{-1} DW; Makus, 1994) and *S. oleracea* (9.9 and 108.6 mg Kg^{-1} DW; Sheikhi & Ronaghi, 2012), as well as *Salicornia* species grown in soils contaminated with metals in European salt marshes (Luque *et al*., 1999; Curado *et al*., 2014; Smillie, 2015).

The contents of N, K and Ca were similar among the progenies of *S. neei*. Their average values were in the same range observed in previous studies with saline effluent irrigation (Bertin *et al*., 2014; Doncato & Costa, 2018). However, *S. neei* progenies had higher values than those observed in shoots of *S. neei* in the salt marshes of Venezuela (Medina *et al*., 2008), and irrigated field with lateritic soil in the semiarid region of Brazil (Alves *et al*., 2020). The concentration of these three minerals ranked in the mid-upper range of values in other species of the subfamily Salicornioideae (Gorham & Gorham, 1955; Lu *et al*., 2010) and gourmet vegetables (Makus, 1994; Sheikhi & Ronaghi, 2012).

CONCLUSIONS

The mineral content of *S. neei* progenies are influenced by irrigation schedule. The extension of the irrigation regime (i.e. T4) provided nitrogen accumulation in shoots, probably associated with the increment of soluble amino-acids under salt/water stress conditions. Lower shoot contents of Ca, Mn and Cu in T4 than T2 were related to element translocation to roots, dilution of elements in larger plant biomass and/or lower loading of saline effluent in T4. Although both tested irrigation schedules might be applied for the cultivation of *S. neei*, extended period between irrigations (i.e. T4) provided the best mineral nutrition profile for human consumption (i.e. gourmet food), since the halophytes presented shoot contents richer in N and with less content of Cu and Mn than T2.

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