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Effect of irrigation salinity and ecotype on the growth, physiological indicators and seed yield and quality of *Salicornia europaea*

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Highlights

- *Salicornia europaea* is a promising agricultural option in coastal desert areas
- Brackish water irrigation (25 dS m^{-1}) optimizes germination, growth and seed yield
- The best ecotype exhibits higher K^+ and $\delta^{15}N$ and lower Na^+ and $\delta^{13}C$ in dry matter
- Irrigation conditions do not affect the fatty acid profile and protein content of seeds

Abstract

The euhalophyte species *Salicornia europaea* is cultivated for oilseed and as a fodder crop in various parts of the world. In saline coastal environments it possesses great potential for the subsistence of the most disadvantaged farmers. We investigated the effect of salinity levels in irrigation water on the germination capacity, shoot biomass and seed productivity as well as diverse quality traits (nitrogen content in shoots and seeds and fatty acids, in seeds) and physiological traits (stable carbon and nitrogen isotopes and ion content) of two accessions collected in the United Arab Emirates (UAE). The three salinity levels tested were irrigation with fresh water (0.3 dS m^{-1}), brackish water (25 dS m^{-1}) and sea water (40 dS m^{-1}). In addition, a hypersaline condition (80 dS m^{-1}) was also tested for germination. The best germination rates were achieved with seeds exposed to fresh and brackish water, while imbibition with sea water decreased germination by half and hypersaline water inhibited it almost totally. However, the best irrigation regime in terms of biomass and seed yield involved brackish water. Moreover, rising salinity in the irrigation increased the stable isotope composition of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), together with the Na^+ and K^+ of shoots and seeds, and the lipid levels of seeds, while the total nitrogen content and the profile of major fatty acids of seeds did not change. Differences between the two ecotypes existed for growth and seed yield with the best ecotype exhibiting lower $\delta^{13}C$ and higher K^+ in both shoots and seeds, lower Na^+ and higher $\delta^{15}N$ in shoots, and lower N in seeds, together with differences in major fatty acids.

Physiological mechanisms behind the response to irrigation salinity and the ecotypic differences are discussed in terms of photosynthetic carbon and nitrogen metabolism.

Keywords: biomass, fatty acids, irrigation, stable isotopic composition, Salicornia, seed.

1. Introduction

Fresh water is a scarce commodity, particularly in the Middle East and other arid subtropical regions of the world where renewable water resources are infrequent and ground water availability is decreasing. The water economy in these regions is beset by a number of problems: high irrigation needs, changes in consumer demands, growing populations and estimated future climatic changes [1]. Despite support irrigation being one of the most efficient agronomical strategies to cope with variability in the present and future climate, such factors may limit the feasibility of conventional agriculture. For this reason, agriculture is exploring novel strategies to harness unconventional water resources and marginal lands [2].

The sandy regions that border the Indian Ocean, the Gulf of California, the Red Sea, and the Arabian Gulf can be used to cultivate halophytes as an oilseed crop [3]. Some of the most productive and salt-tolerant halophytes are shrubby species of the *Salicornia* genus, and some members can be grown successfully in arid regions using sea water for irrigation and have been proposed as oilseed and fodder crop in various parts of the world [3,4]. In saline coastal environments they may represent a means of subsistence for the most disadvantaged farmers or as an alternative for areas where no other crops will grow. However, while the suitability of species such as *Salicornia bigelovii* Torr. has been quite extensively investigated [5-8] other species have had comparatively little attention.

European Salicornia (*Salicornia europaea* L.) is a halophyte species that grows in areas periodically flooded by salty or brackish tidal water. In fact, as one of the most salt-tolerant plant species in the world, *S. europaea* can tolerate more than 1000 mM NaCl, and optimal growth and photosynthetic rates have been observed following treatment of the plant with 200-400 mM NaCl [9]. European Salicornia is edible, either raw or cooked. In addition, it can be used as a pasture for animals, as a biofuel, and has potential in pharmaceuticals and cosmetics [10,11]. It also has a high fatty acid content in its seeds and their characteristics make them a nutritional source of great interest [12,13]. Recent studies at the International Center for Biosaline Agriculture (ICBA) in the United Arab Emirates (UAE) show that some varieties of *S. europaea* cultivated with good agronomic practices grow well in marginal soils and can be economically viable.

To contribute to a wider adoption of this species as a crop it is important to develop improved varieties alongside optimization of the agronomical conditions to ensure higher biomass and/or seed yield and quality. With regard to genetic improvement, a strategy to be pursued is to use ecotypes (i.e. wild varieties) already well adapted to the target cultivation environment as starting germplasm [7]. Concerning agronomy, determining suitable levels of salinity during irrigation for the best germination, growth, seed yield and quality outcomes is paramount. Additionally, a better understanding of the genetic

and environmental influence on seed oil fatty acid composition would be helpful in developing *S. europaea* as a viable crop for salinity-affected environments.

The stable carbon and nitrogen signatures of plants in their natural abundance may help to better understand how the carbon and nitrogen metabolism of plants responds to salinity [14]. Thus the carbon isotope composition ($\delta^{13}C$), when analysed in dry matter, provides a time-integrated indication of the intercellular to atmospheric CO_2 ratio (Ci/Ca) achieved by the plant during photosynthesis [15]. Ci/Ca reflects the balance between stomatal conductance and the intrinsic photosynthetic capacity of the plant. Therefore, Ci/Ca may be affected in the short term by the water stress component of salinity (closing stomata), while in the long term the ionic component (affecting photosynthetic metabolism) of the salinity may also affect this ratio. In fact, $\delta^{13}C$ has been extensively used to assess plant responses to salinity (e.g. [16]), including halophyte species such as *S. europaea* [17-19]. Thus, it is expected that $\delta^{13}C$ values increase (become less negative) in response to salinity stress. However, to the best of our knowledge, the use of $\delta^{13}C$ to assess genotypic differences in the *Salicornia* genus has not been reported. In this sense it is worth exploring the feasibility of $\delta^{13}C$ as an indicator of the effect of growing conditions (differences in biomass, yield, transpirative flow) and genetic diversity on the performance of this halophyte species

The nitrogen isotope composition ($\delta^{15}N$), together with the total N content in plant matter, give insights on the effect of salinity and genotype on the nitrogen metabolism of the plant [20,21]. For example, it has been reported for non-halophyte herbaceous plants (like cereals) that salinity decreases the nitrogen content in tissues as well as the $\delta^{15}N$, and frequently a positive relationship between $\delta^{15}N$ and biomass/yield has been reported [14,16]. However, no studies on the effect of salinity on the $\delta^{15}N$ of halophyte species from the Salicornia genus have been reported.

The objective of this study was to investigate the effect of salinity levels in the irrigation water, on the germination capacity, plant growth (biomass) and seed productivity, as well as the quality of the latter in terms of fatty acids, total proteins and ion content. In addition, we investigated the physiological response of salinity levels during growth by analysing the $\delta^{13}C$ and $\delta^{15}N$ as well as the content of nitrogen and ions in stems and seeds. We worked with two ecotypes of European Salicornia, native to coastal areas [22] of the UAE.

2. Materials and methods

2.1 Plant material

Two ecotypes of *Salicornia europaea* L. were assayed. These are local populations that grow naturally on the coastal shores of the Emirates of Ra's al-Khaimah (RAK) and Umm al-Quwain (UAQ) of the UAE (Fig. 1). Seeds of both ecotypes were collected in situ in 2016 and then multiplied in a greenhouse using brackish water (25 dS m^{-1}) at the International Center for Biosaline Agriculture (ICBA), Dubai, UAE (25°05′49″ N and 55°23′25″E). At harvest, mature seeds of both ecotypes were collected randomly from across the whole population to represent its genetic diversity. Immediately after

collection, the seeds were air dried and threshed to separate debris. Seeds were cleaned and stored in brown paper bags at 4°C until use in the growth experiments.

2.2. Plant growth and growing conditions

For this study the two ecotypes of *Salicornia* were grown in pots (16 l) on a substrate formulated with two parts sand and one part manure compost in a greenhouse at the ICBA facilities. Details about the composition of the compost are reported elsewhere [23]. About 25 seeds were planted in each pot. Eight weeks later the plants were thinned, leaving three plants per pot.

Three levels of water salinity (measured as electric conductivity, EC) were evaluated during irrigation: 0.3 dS m^{-1} (fresh water), 25 dS m^{-1} (simulating brackish water from the water table) and 40 dS m^{-1} (sea water). The ion concentrations of the different water sources used for irrigation are detailed in Table 1. The respective water treatments (0, 25, 40 dS m^{-1}) were started at the time of sowing (first day). The experiment was laid out in a complete randomized block design. For each ecotype and growing condition, 36 pots were used. For irrigation, 1 l of water was given to each pot daily.

Sowing took place on Jan 4, 2017 and plants were harvested the second week of December 2017. Environmental conditions inside the greenhouse are shown in Supplemental Figure 1.

At harvest, for each ecotype and growing condition a subset of five pots from the central part of the total of 36 pots were processed. For each pot all shoots were collected, washed with tap and distilled water, dried at 40 °C in an oven for 48 h and then threshed to remove the seeds. Then the two components (shoots and seeds) were weighed separately and expressed in grams per plant. Seeds from plants grown under the three irrigation salinity levels were cleaned and stored in plastic bags at 4°C until use in the germination experiment. In addition, for each ecotype and growing condition, shoots and seeds were ground to a fine powder, dried in an oven at 60 °C for 48 h and then used for the subsequent analyses.

2.3. Ion analyses

From each sample, 100 mg of the ground dry material (either shoots or seeds) was digested with 3 ml of concentrated HNO_3 and 2 ml of H_2O_2, then heated overnight at 90 °C. Afterwards, 30 ml of distilled water was added to each sample and then transferred to analytic tubes. The amount of Na^+, K^+, P, Ca^{2+} and Mg^{2+} in each sample was determined using an Inductively Coupled Plasma Emission Spectrometer (L3200RL, Perkin Elmer, Uberlingen, Germany) at the Scientific Facilities of the University of Barcelona. Ion concentrations were expressed in mmol g^{-1} of dry weight.

2.4 Stable carbon and nitrogen isotope composition and nitrogen content

A second subsample of the dry leaf powder was used for the measurement of the total nitrogen concentration and the stable carbon ($^{13}C/^{12}C$ ratio) and nitrogen ($^{15}N/^{14}N$ ratio) isotope signatures. Before the analyses, in order to eliminate the lipid contents from seeds, samples were dissolved in methanol (50mg ml^{-1}) and centrifuged threefold (5 min, 25 °C,

12000 rpm). The supernatant was then discarded and the pellet dried at 60 °C for 48h. Analyses were performed at the Scientific Facilities of the University of Barcelona. Samples of ~1 mg were weighed into tin capsules and analyses were performed using an elemental analyser (Flash 1112 EA; ThermoFinnigan, Schwerte, Germany) coupled with an isotope ratio mass spectrometer (Delta C IRMS, ThermoFinnigan), operating in continuous flow mode. The $^{13}C/^{12}C$ ratios (R) of plant material were expressed in δ notation [24] as carbon isotope composition ($\delta^{13}C$): $\delta^{13}C(‰) = \left[\left(R_{sample} / R_{standard}\right) - 1\right] x\ 1000$, where, sample refers to plant material and standard to Pee Dee Belemnite (PDB) calcium carbonate. International isotope secondary standards of known $^{13}C/^{12}C$ ratios (IAEA CH7 3, polyethylene foil; IAEA CH6, sucrose; USGS 40, L-glutamic acid) were used with an analytical precision of 0.1‰. The same δ notation was used for the $^{15}N/^{14}N$ ratio expression, but with the standard referring to N_2 in air. For nitrogen, the international isotope secondary standards IAEA N1, IAEAN2, IAEANO_3, and USGS40 were used with a precision of 0.3‰.

2.5. Fatty acid analysis

The total lipid was extracted from the seeds using a solvent extraction method [25]. The pulverized samples (50mg each) were first dissolved in 5 ml of dichloromethane:methanol (2:1; v/v), stirred for one minute using a vortex, further sonicated for 10 minutes, and then centrifuged threefold for five minutes each. For each centrifugation, 5 ml of solvent was used, and in the second and third centrifugations the pellet was re-suspended. The resulting 15 ml of supernatant was then transferred to a pre-weighed vial and then the solvent evaporated and the vial weighed again. The total lipid content was then calculated from the difference in weight.

For the subsequent analysis of fatty acids, lipid acid extraction followed the same protocol, except that some standards (see below) were added. The supernatant was then transferred to a new test tube and dissolved in 5 ml of 1 M potassium hydroxide in methanol (KOH MeOH), and then saponified overnight. The next day the suspension was extracted with n-hexane to remove neutral lipids. The methanolic phase was then acidified to pH 2 with extraction of free fatty acids using n-hexane, then dried to 1 ml using a rotary evaporator and dissolved in 5 ml boron trifluoride-methanol (BF3 2 M solution in MeOH) overnight (methylation). On the third day, the fatty acid methyl esters obtained were extracted with n-hexane after addition of water. Finally, the extract was dried under a stream of nitrogen and resuspended in a known volume of n-hexane for analysis by gas chromatography (GC)-mass spectrometry. The analytical procedure was performed at the Scientific and Technological Centers of the University of Barcelona.

Fatty acid determination was assessed with standard gas chromatography-mass spectrometry using a GCMS-QP2010 (Shimadzu; Kyoto, Japan). The chromatographic oven was set to initial and final temperatures and hold times of 60°C for 1 min and 120°C for 10 min, respectively, an injection temperature of 260°C, the split injection mode and a constant column flow of helium of 1ml min^{-1}. The spectrometer had an ACQ-scanning mode and ion injection function ranging from 50 m z^{-1} to 650 m z^{-1}. Retention time, peak

area and the ion ratio were compared to FAME (fatty acid methyl ester) GC standards for FA determination.

2.6. Effect of salinity on seed germination capacity

The experiment was carried out in June 2018 at the ICBA facilities. Germination was tested in seeds derived from plants grown under the three different levels of irrigation salinity stated above (EC of 0.3, 25 and 40 dS m^{-1}), using water with four different ECs: 0.3 dS m^{-1} (as control), 25, 40 and 80 dS m^{-1}. The three different saline water treatments were formulated using NaCl. Therefore, a total of 24 treatments (combining ecotype, seed provenance and water EC for germination) were undertaken. For each treatment, three replicates were performed. For each replicate, one hundred seeds were counted using a seed counter (Contador Electronic Seed Counter, Pfeuffer, Germany) and then weighed. Seeds were placed evenly into a 9 cm diameter Petri dish lined with Rundfilter 90 mm filter paper saturated daily with the required amount of control irrigation or the relevant treatment solution. The dishes were kept under a photoperiod of 16h/8h (light/ darkness) at 25±2°C for 9 days with a relative humidity of 70%. Afterwards, seed germination was measured, with germination defined as extension of the radicle by at least 2 mm.

2.7 Statistical analyses

Analysis of variance (ANOVA) was performed using the statistical package SPSS (SPSS Inc., US) to test the effects of the irrigation treatment, ecotypes, and their interaction. A bivariate correlation was carried out also using SPSS software to calculate Pearson correlation coefficients between the analytic traits and yield components. A multiple linear regression using the stepwise procedure was conducted to extract the significant traits that explain biomass and yield variations across treatments. Principal components analysis was performed using the statistical software R (RStudio, Inc.), to analyse yield components and all analytical traits on a bi-dimensional plan and classify ecotypes for each studied environment. Graphs were created using the SigmaPlot 10.0 program (Systat Software Inc., US).

3. Results

3.1. Plant growth

The effect of irrigation salinity on seed weight per plant, together with shoot dry matter and other plant growth traits (plant height, number of branches per plant and number of inflorescences per plant), were evaluated in the two ecotypes (Table 2). Seed yield and shoot biomass per plant, together with the other growth traits per plant, were favoured by treatment with brackish water (25 dS m^{-1}), and except for seed yield in the RAK ecotype, these traits were still somewhat higher with the sea water treatment (45 dS m^{-1}) relative to freshwater irrigation. Thousand seed weight (TSW) decreased from fresh to brackish water. Seed yield, shoot biomass and the other agronomical traits expressed per plant were significantly higher in the UAQ ecotype than RAK, whereas TSW exhibited the

opposite relationship. Except for plant height and TSW, no genotype by environment interaction was recorded.

3.2. Stable carbon and nitrogen isotope composition and total carbon C and N content

The level of salinity did not affect the concentration of nitrogen in the seed, while the nitrogen in the shoot decreased slightly (Table 3). Regardless of the salinity conditions, nitrogen values were more than ten times higher in seeds than in the mature shoots. The carbon concentration in both shoots and seeds decreased as salinity increased, with values in seeds being around 50% higher than in shoots. The nitrogen isotope composition ($\delta^{15}N$) increased in both shoots and seeds in response to the level of salinity in the irrigation, while the values in seeds were higher than in shoots. The carbon isotope composition ($\delta^{13}C$) also increased (became less negative) in response to the increase in salinity, and again the values in the seeds were consistently higher than in the shoots. Differences between ecotypes were significant except for the nitrogen concentration in the shoots and the $\delta^{15}N$ of seeds. Carbon concentrations in the shoots and seeds and the $\delta^{15}N$ in the shoots were higher, while the nitrogen concentration of the seeds, together with the $\delta^{13}C$ of both the shoots and seeds, were lower in UAQ than RAK. No interaction of ecotype by irrigation conditions was observed for any of the traits presented in Table 3.

3.3. Ion concentration

Higher salinity in the irrigation water increased the concentration of Na^+, K^+ and Mg^{2+} and reduced P in the shoots, while the concentration of Ca^{2+} was maximal when plants were irrigated with brackish water (Table 4). Concerning the seeds, higher salinity in the irrigation water increased the concentration of Na^+, and to a lesser extent K^+, and reduced the P concentration, while the effect on Ca^{2+} and Mg^{2+} concentrations was not significant. The UAQ ecotype exhibited higher concentrations of Na^+ and K^+ in the shoots and lower concentrations of Ca^{2+} and P than in the RAK ecotype, while no differences between ecotypes existed for Mg^{2+}. Concerning seeds, the concentration of K^+ was consistently higher while P and Mg^{2+} were lower in UAQ than RAK and no differences existed for Na^+ and Ca^{2+}. The interaction between ecotype and salinity level in the irrigation water was only significant for K^+ in both shoots and seeds.

The Na^+/K^+ ratio in shoots was higher in RAK compared to the UAQ genotypes, regardless of the growing conditions. Moreover, while it remained more or less steady in RAK as salinity increased, the Na^+/K^+ ratio decreased in UAQ at the highest salinity level. In seeds the Na^+/K^+ ratio was also higher in RAK than UAQ, but in both genotypes the values increased as salinity levels increased.

3.4. Relationship between yield, growth and physiological traits

The overall relationships of the $\delta^{13}C$ and $\delta^{15}N$, the carbon and nitrogen contents, and the different ions in the shoot and seeds with the shoot biomass and seed yield were assessed within each irrigation treatment through a principal component analysis (PCA). For this purpose, the data for each ecotype within each irrigation regime were pooled. For the three irrigation treatments, the first two components explained around 60% of the total

variance (Fig. 2). For all three growing conditions, seed yield, shoot dry weight, plant height and the number of branches per plant were located around the first axis (PC1). In the case of irrigation with fresh water (FW), the parameters closest to seed yield and the growth traits were shoot $\delta^{15}N$ and K^+ and seed Na^+ and K^+, while shoot P and seed $\delta^{13}C$, N, Mg^{2+} and P were on the opposite side of the PC1 axis. Under brackish water, shoot biomass, seed yield, plant height and branches per plant were placed near seed K^+ and shoot K^+ and opposite to shoot Na^+ and Ca^{2+} and seed $\delta^{13}C$, N and P. Under sea water), seed yield, plant height, branches per plant, shoot biomass, along with shoot K^+ and seed K^+ and C were placed in the right hand side of the figure. Shoot $\delta^{13}C$ and Na^+ and seed $\delta^{13}C$, $\delta^{15}N$ and N were located on the opposite side. On the other hand, the two ecotypes were clearly differentiated within each salinity level, with UAK placed closer to shoot biomass, seed yield and agronomical traits per plant than the RAK ecotype (Fig. 2).

Placing together data from both ecotypes and the three salinity conditions the pattern of the relationships of seed yield with both seed $\delta^{13}C$ and shoot Na^+ was curvilinear, with seed yield values being achieved at intermediate $\delta^{13}C$ and Na^+ values (Supplemental Fig. 2). A comparable but slightly weaker pattern was found when relating seed yield to shoot $\delta^{13}C$ and seed Na^+. For each ecotype, shoot $\delta^{13}C$ was positively correlated with shoot biomass across the three irrigation conditions. The linear relationship was in line with the RAK ecotype exhibiting consistently higher $\delta^{13}C$ for a given shoot biomass (Fig. 3). In addition, for each ecotype, shoot $\delta^{13}C$ was positively linearly correlated with shoot Na^+ content (Fig. 4). The slope of the UAQ fitted line was lower than the RAK ecotype line, with Na^+ values being increasingly higher in RAK than UAQ as $\delta^{13}C$ increased. In addition, for each genotype, $\delta^{13}C$ was positively correlated with $\delta^{15}N$ across growing conditions, with both lines indicating that the UAQ ecotype exhibited higher $\delta^{15}N$ than RAK for a given $\delta^{13}C$ value (Fig. 5).

The growth parameter that best correlated with the physiological parameters analysed within each salinity level was the number of branches per plant. Thus, this agronomic parameter was correlated negatively with ionic contents, especially of the seeds at the highest salinity, and also with the $\delta^{13}C$ of the seeds in two of the salinity levels and correlated positively with $\delta^{15}N$ in one of the levels (Table 5). However, the parameter that correlated best in the three levels of salinity, was the nitrogen content of the seeds, but the correlations were negative (Fig. 6), which suggests that there is a balance between growth (at least for branching) and seed quality in terms of protein content.

3.5. Concentration of fatty acids in seeds

The effects of salinity conditions and ecotype on the total lipid content as well as the relative content of fatty acids in the seeds were studied (Table 6). Irrigation had a significant effect on the total lipid content. Increasing salinity in the irrigation from fresh to brackish water increased the total lipid content, whereas when sea water was used, the lipid content tended to decrease (Table 6). Except for RAK under fresh water, the total lipid content ranged between 32 and 36%. No differences between ecotypes were observed.

The relative composition of fatty acids in the seeds in response to ecotype and salinity effects was also studied (Table 6). The most abundant fatty acid was n6c linoleic acid

(accounting for around 60%), followed by n9c oleic acid (close to 20%), palmitic acid (above 10%), alpha linolenic acid (near 5%) and stearic acid (around 2%), together comprising more than 95% of the total fatty acid content. The fraction of unsaturated plus polyunsaturated fatty acids represented more than 85% of the total fatty acids.

Ecotype differences existed for the main fatty acids except in the case of palmitic acid. Compared with UAQ, RAK ecotypes exhibited higher n6c linoleic and stearic acid contents and lower alpha linolenic and n9c oleic acid contents. Differences between ecotypes also existed for a few other minor fatty acids, such as pentadecanoic, palmitoleic, margaric and cis-10-heptadecenoic acids. By contrast, salinity conditions did not affect the relative content of major fatty acids and a significant G x S interaction was only found for alpha linolenic acid.

3.6. Germination ability

We carried out a test of the effect of water salinity on germination and its interaction with the provenance of the seeds. Specifically, germination of seeds produced from plants grown under four different salinity levels was tested under three different salinity levels (Table 7). Germination was very similar in fresh water (0.3 dS m^{-1}) and brackish water (25 dS m^{-1}), with values around 60%, but it decreased by half at salinity levels comparable to sea water (40 dS m^{-1}) and fell almost to zero under hypersaline conditions (80 dS m^{-1}). The growing conditions during seed production also affected germination in the next generation. Indeed, when germinated under fresh and brackish water, seeds from sea water grown plants exhibited somewhat lower germination (around 20%) than seeds from plants grown under fresh and brackish water. By contrast, no differences among growing conditions were detected when germination took place with sea water. In fact, the interaction between salinity conditions during growth and during germination was significant. No significant differences were observed between the ecotypes, and the interaction between ecotype and water salinity during germination was not significant. However, the interaction between ecotype and salinity during growth almost reached significance (P=0.059) and the same occurred for the triple interaction (salinity during growth x salinity during germination and ecotype) (P=0.055).

4. Discussion

4.1. Salinity conditions and plant growth

In keeping with its halophyte characteristics, the growth (in terms of plant height, number of branches, inflorescences and shoot biomass) and seed production of the euhalophyte *Salicornia europaea* was favoured with brackish water (25 dS m^{-1}), and growth traits were still higher with sea water (40 dS m^{-1}) with respect to fresh water. Maximal growth and photosynthetic rates have been reported under saline conditions formulated with NaCl, within the range 15-40 dS m^{-1} [9,26] but *S. europaea* can withstand more than 1000 mM NaCl and is one of the most salt-tolerant plant species in the world [27,28]. Moreover, in the absence of salinity it has a tendency towards lower biomass than under saline conditions [29]. In the present study the seed yield per plant and the thousand seed weight (TSW), were in the range of values reported before in *Salicornia spp*. [30].

Moreover, the TSW slightly decreased in response to salinity, and was associated with an increase in the number of seeds under saline conditions. The decrease in the TSW as salinity increased may have complementary explanations. *S. europaea* produces dimorphic (larger and smaller) seeds that differ in size and plant position [31,32]. While the relative importance of each category of seeds on the plant may be related to the architecture of the shoot, our study clearly found that the number of branches and inflorescences per plant increased in response to salinity, particularly when comparing fresh water to brackish water (Table 2).

4.2. Salinity conditions: ion accumulation and $\delta^{13}C$

As irrigation salinity increased, Na^+ accumulation in the shoots was much higher than K^+ and the other two cations analysed (Ca^{2+} and Mg^{2+}), which agrees with the halophyte nature of this species. Without salt glands or salt bladders, *S. europaea* plants can accumulate considerable amounts of Na^+ in their shoots, even up to 50% of the dry weight [28,33]. The values in our study would be between 5% of the total dry weight [32] and 20-30% of the dry weight [9,34]. Even under irrigation with fresh water the amount of Na^+ accumulated in the shoots was around ten times higher (UAQ ecotype) or even more (RAK ecotype) than the levels of K^+. Moreover, Na^+ was the main ion accumulated in the seeds. Indeed, when both ecotypes were grown with fresh water, the Na^+ content in the seeds was several times higher than the K^+ content. While the euhalophyte nature of *S. europaea* may favour the accumulation of Na^+, the manure used for soil amendment might have influenced the high level accumulation of this ion under freshwater conditions. While the use of manure may be appropriate for increasing the organic matter in sandy soils in arid regions, the amount and particular nature of the manure used in Dubai, which is rich in Na^+ and other ions, represents a significant source of salinity [23].

The range of carbon isotope composition ($\delta^{13}C$) values in shoots and seed dry matter and their response to salinity, even when irrigated with sea water, clearly corresponded to plants with C_3 metabolism [15], and *S. europaea* is known as a C_3 succulent species [35]. Comparable $\delta^{13}C$ values have been reported before in the same species [36], with values increasing (becoming less negative) up to 8 ‰ [17,19] as soil water potential decreased in response to salinity from 0 to near -8 MPa.

In the case of non-halophyte C_3 species, the ^{13}C enrichment, and consequently the increase in $\delta^{13}C$ under increased salinity may be a result of stomatal closure, with the subsequent decrease in the intercellular to atmospheric CO_2 concentration (C_i/C_a) ratio [15] being due to water stress [16,37]. However, in the case of a C_3 halophyte species such as *S. europaea*, the way in which increasing salinity reduces the C_i/C_a ratio (therefore causing an increase in $\delta^{13}C$) may be different. It is most likely the consequence of a biphasic process, characterized as an increase in internal photosynthetic capacity as salinity increases from fresh water to brackish water, while further increases in irrigation salinity to levels found in sea water result in decreases in stomatal conductance. The pattern of reported gas exchange (photosynthetic rates and stomatal conductance) responses in *S. europaea* subjected to increasing salinity supports such a conclusion. In fact, net photosynthesis and stomatal conductance in *S. europaea* have been reported to increase

as salinity increases from fresh water to around 20 dS m^{-1}, then decreases from 40 dS m^{-1} to values still slightly higher than under fresh water conditions [9]. Earlier studies on gas exchange in this species also support this pattern [38]. In other species of *Salicornia*, such as *S. fruticosa*, the decline in photosynthetic rates at salinity levels beyond brackish water also originates from decreased conductance [39].

The positive effect of salinity on gas exchange in *S. europaea* when transitioning from irrigation with fresh water to brackish water may be related to the role of shoot Na^+ as an osmoticum. It has been reported that Na^+ in *S. europaea* cells may act as an effective osmotic adjuster to maintain cell turgor and relative water content, promoting photosynthetic competence and plant growth, with Na^+ being compartmentalized predominantly into the cell vacuoles of shoot endodermis tissues [9]. In our study, Na^+ in shoots was strongly positively correlated with shoot $\delta^{13}C$ across the different salinity levels (Fig. 4), while changes in the other cations correlated more weakly (K^+, Mg^{2+}) or did not correlate (Ca^{2+}) with shoot $\delta^{13}C$. Unlike previous work [29,40], even though Na^+ was the main cation that accumulated as irrigation salinity increased in our study, other cations like K^+ and Mg^{2+} also increased, and even Ca^{2+} showed no decline as irrigation salinity increased. Nevertheless, considering the relative amount of each ion, Na^+ may act as the main effective osmotic adjuster [40], promoting photosynthetic competence and plant growth [9,34]. In any case, a decrease in stomatal conductance at very high salinity levels could reduce the flux of salt into the shoot and in fact in our study shoot accumulation of Na^+ was not lineal in relation to the increase in irrigation salinity (Table 4). These results agree with the linear negative correlation between $\delta^{13}C$ and soil potential reported for *S. europaea* grown under different salinity levels [19, 40].

In support of the potential role of K^+ in the response to increases in salinity in *S. europaea*, the K^+ content increased while the Na^+/K^+ ratio in shoots decreased as salinity increased. This result contrasts with previous reports of the response of the same species to increasing levels of NaCl or other chloride salts during growth [29,32]. However, when sulfate salts were used instead, increasing salinity conditions had a minor effect on the K^+ levels [32]. In the growing conditions of our experiment, the addition of manure to the soil ensured the presence of sulfate salts.

4.3. Salinity conditions: nitrogen concentration and $\delta^{15}N$

The nitrogen concentration in shoots was very low, being around half the value reported previously in *S.* europaea [26]. This is because we collected shoots at maturity, whereas the values reported by Reiahisamani et al. [26] corresponded to vegetative tissues collected between 2 and 3 months after germination. Under increased salinity there was a decrease in the N concentration in shoots, and a similar response has been reported in non-halophyte species [14,16], although the mechanisms behind this are probably different. In the case of *S. europaea*, a dilution effect associated with greater growth together with Na^+ accumulation may be involved. Additional factors such as an increase in sclerenchyma tissues and succulence under saline conditions may also occur [41,42]. In support of an effect due to Na^+ accumulation and change in anatomy, the total carbon content also decreased in response to salinity. Differences relative to non-halophyte species in the effect of salinity on nitrogen metabolism are supported by the increase in

nitrogen isotope composition ($\delta^{15}N$) as salinity increased and the positive relationship between $\delta^{15}N$ and a $\delta^{13}C$ (Fig. 5) in *S. europaea*. The opposite pattern has been reported for non-halophyte species such as durum wheat [16,43], barley [44] and quinoa as salinity increases.

In non-halophyte species, besides inducing stomatal closure and therefore increasing $\delta^{13}C$, salinity also affects processes related to N uptake, assimilation, release and internal recycling, and consequently decreases plant $\delta^{15}N$ [14,45]. However, in *S. europaea* the increase in $\delta^{15}N$ and the positive relationship between $\delta^{15}N$ and $\delta^{13}C$ should have been sustained by mechanisms associated with a higher stomatal conductance, transpiration and growth as salinity increased. Thus, the $\delta^{15}N$ in leaf metabolites is predicted to depend on the reduced N input [46]. Typically, glutamic acid and glutamine are expected to be more ^{15}N-enriched when the N input is proportionally lower [46]. Such a situation is believed to occur, for example, in the absence of abiotic stresses (which in the case of halophyte species means growing under saline conditions). This would be associated with a low balance of N availability versus uptake and assimilation by the plant [47-49], facilitated by higher activities of key N-metabolism enzymes like nitrate reductase and glutamine synthase [14,20] and associated in our study with an increase in growth as salinity increased. In addition, other fractionation processes may occur during the export of nitrogen-containing compounds from the roots to the shoots [21,50], or an increase in the loss of ammonia and nitrous oxide due to a higher stomatal conductance [51,52], which again may be the situation in our study, at least when transitioning from fresh water to brackish water irrigation.

4.4. Ecotypic differences in performance

The UAQ ecotype grew more and produced greater biomass and seed yield than the RAK ecotype. Ecotypic differences in biomass have been reported before for *S. europaea* [26], but the physiological mechanisms behind them have not been considered. In our study, for a given salinity level the UAQ ecotype exhibited lower $\delta^{13}C$ values in shoots and seeds than the RAK ecotype. Therefore, for a given $\delta^{13}C$ value, shoot biomass and seed yield were higher in UAQ than RAK. Since shoot $\delta^{13}C$ values in *S. europaea* have been reported as being strongly negatively correlated with osmotic potential conditions [17,19], this suggests that UAQ may be better at sustaining photosynthesis than RAK, which is likely due to increased stomatal opening, and this results in a higher growth rate. In that sense, Munns and James [53] concluded that screening for high stomatal conductance may be the most effective way of selecting genotypes for rapid growth in saline conditions. However, such a pattern of maintaining higher stomatal conductance does not necessarily characterize genotypic tolerance to long-term exposure to salinity in non-halophyte species [14,16].

However, the Na^+ concentration in shoots tended to be higher in RAK than UAQ and the absolute differences between the ecotypes increased as salinity levels increased. In the same way, as $\delta^{13}C$ increased, differences in shoot Na^+ between the ecotypes increased, with UAQ exhibiting lower Na^+ values than RAK. Therefore, these results do not support the concept that Na^+ played an osmotic role in the better performance of UAQ. The

concentrations of other elements such as Ca^{2+} and P in the shoots were also higher in RAK than UAQ. Nevertheless, the higher total nitrogen and Na^+ contents in shoots of RAK relative to UAQ might simply have been a consequence of a lower dilution effect in the former due to its smaller shoot biomass. By contrast, irrespective of the salinity conditions, more K^+ accumulated in UAQ shoots and seeds of the than in the RAK genotype. Moreover, in the former the K^+ rose much more in the shoots as salinity increased, than in the less productive RAK. This suggests that even in a halophyte like *S. europaea*, K^+ accumulation may have a positive role. Thus, a multiple sodium compartmentalization mechanism has been proposed for this species that further enhances salt tolerance via Na^+ sequestration into the vacuoles [9]. As salinity conditions increase, a rise in the Na^+ concentration accumulated in the vacuole is expected and therefore a substantial osmotic potential gradient would be established between the vacuole and the cytosol by depressing the cytosol's water activity. However, this change requires a coordinated increase in compatible solutes in the cytosol, such as K^+, to balance out the changes in osmotic pressure in the salt-stressed plants [37]. Moreover, maintaining cytosolic K^+ concentrations are essential for plant metabolism, including stomatal opening and photosynthetic activity, while vacuolar K^+ concentrations may vary dramatically [54,55]. Therefore, a high cytosolic K^+/Na^+ ratio is essential for maintaining good physiological status under saline conditions [56]. In our study, regardless of the salinity conditions, the best performing ecotype exhibited a higher K^+/Na^+ ratio even when analysed in terms of the shoot dry matter. Moreover, there is increasing evidence of cell- and tissue-specific signalling roles for K^+ during plant salt tolerance [57]. All of these results contrast somewhat with the study of Lv et al. [34], which concluded that Na^+ plays a more important role in the growth and development of *S. europaea* than K^+. However, Na^+ is not the only major vacuolar osmoticum in *S. europaea*, glycine betaine is also involved and its quantities increase with salinity [29]. Nevertheless, our results are in line with K^+ playing a positive role in the ecotypic differences observed. Besides its role as an osmoticum, K^+ has a positive role in xylem transport and stomatal opening, which may contribute to wider stomatal apertures (and thus lower $\delta^{13}C_{shoot}$) and higher photosynthetic rates in the UAQ compared to RAK. The consistently more negative $\delta^{13}C$ in the shoots and seeds of UAQ relative to RAK across the different irrigation conditions supports a higher stomatal conductance in the former.

The effect of K^+ in xylem transport may also affect shoot $\delta^{15}N$. Thus, $\delta^{15}N$ can be used as a natural tracer of N fluxes from the substrate through the plant. It is believed that after having been absorbed, non-assimilated nitrate is ^{15}N-enriched due to nitrate reduction in roots, and therefore nitrate molecules that are transferred to the shoots via the xylem are naturally ^{15}N-enriched [20,58]. Thus, lower K availability may down-regulate sap circulation, diminishing the leaf / shoot enrichment in ^{15}N and then causing lower increases in $\delta^{15}N$ [21]. In our study and in agreement with its higher growth and K^+, the UAQ ecotype exhibited higher $\delta^{15}N$ in the stem than RAK. A number of other explanations may account for the higher $\delta^{15}N$ and lower $\delta^{13}C$ of UAQ relative to RAK in response to salinity. Thus, higher stomatal conductance in the UAQ would lead to a greater loss of ammonia and nitrous oxide, hence increasing the $\delta^{15}N$ of the dry matter [51,52]. Alternatively, a lower rate of photorespiration in UAQ (associated with higher stomatal conductance) does not seem to be involved in the ecotypic differences in $\delta^{15}N$ because this process would go in the opposite direction, decreasing the $\delta^{15}N$ in N-

metabolism precursors [46,58]. Moreover, for each ecotype, shoot $\delta^{13}C$ and $\delta^{15}N$ were positively correlated across salinities (Fig. 5), with UAQ exhibiting higher $\delta^{15}N$ than RAK for a given $\delta^{13}C$ value. Therefore, factors other than stomatal conductance might also be involved in the ecotypic differences in $\delta^{15}N$.

In the case of non-halophyte species, the differences in $\delta^{15}N$ between salinity tolerant and susceptible genotypes suggest that the genotypic tolerance to salinity is mediated through a higher N uptake and/or assimilation in the former [49,59]. Nevertheless, in contrast to reports on non-halophyte species [14,16,43], in the current study the most tolerant ecotype did not show higher N content on a dry matter basis. These results do not support a lower demand for N in RAK, the ecotype with the lower rate of growth, and this led to stress-induced depletion in plant ^{15}N [47].

4.5. Seed quality

The nitrogen concentration in seeds did not change, whereas phosphorus decreased as irrigation salinity increased. The nitrogen content was very high and corresponds with the high protein content (42–45%) reported for other Salicornia species like *S. bigelovii* [5]. The seeds of UAK, the best performing ecotype, exhibited lower N and P concentrations than the RAK ecotype. These differences may be the result of a dilution effect in the former ecotype because of its higher seed yield. Interestingly, when combining the data from both ecotypes, a strong negative relationship was found within each level of irrigation salinity between N concentration in seeds and the number of branches per plant (Fig. 6), a strong trait that determines final seed yield.

Concentrations in seeds of Na^+, and to a lesser extent K^+, increased as the salinity conditions increased, despite the opposite trend of an increasing Na^+/K^+ ratio in the shoots, and this reflected preferential accumulation of Na^+ in seeds. Nevertheless, when grown with sea water, the total salt content in the seeds did not reach 2% of the dry weight, and was in the range of (or even lower than) levels reported for other Salicornia species, such as *S. bigelovii*, which is amenable to sea water cultivation [3]. However, in line with the greater accumulation of K^+ in UAK shoots than RAK shoots, the K^+ in seeds was higher in the former, while no differences in the Na^+ concentration was found between the genotypes.

The oil content of the seeds was high (in general, slightly higher than 30% of the seed biomass), and in the range of a number of reports on *S. europaea* [12,26] and other species such as *S. bigelovii* [3], but near double other data from *S. europaea* [13]. Moreover, the oil content increased as salinity conditions increased, but while we found the highest values when irrigating with brackish water (Table 6), previous studies have reported 600 mM NaCl (i.e. values around sea water) as the optimal conditions for oil content in seeds [26].

The relative composition of fatty acids in the seeds as a function of ecotype and salinity effects were also studied (Table 6). The most abundant fatty acid was n6c linoleic acid (accounting for around 60%), followed by n9c oleic (near 20%), palmitic (above 10%), alpha linolenic (near 5%) and stearic (around 2%) acids, together accounting for more than 95% of the total fatty acid content. The relative quantities and importance of major fatty acids were similar in a recent report on Iranian ecotypes of *S. europaea* [26] with

only alpha-linolenic and stearic acids showing some differences in order (fourth and fifth in our study and the reverse in the Reiahisamani *et al.* paper). Studies in other *Salicornia spp.* have also identified these five fatty acids as the most abundant, together accounting for nearly 95% of the total fatty acid content, with alpha-linoleic (29%), followed by palmitic (25.8%) and stearic (25.7%) acids comprising 80% of the total [60]. This contrasts with another publication on *S. europaea* were oleic acid was the most abundant fatty acid, even though its relative content was similar to our study [13]. However, this previous study delivered quite surprising results, with nervonic (16.5%), eicosenoic (14.3%), palmitoleic (12.7%), alpha-linolenic (10.6%) and palmitic (6%) acids being the most important, whereas linoleic acid was not detected.

Our study, with ecotypes collected in the UAE, identified differences in the major fatty acids (except palmitic acid), which agrees with a previous study where differences were reported across Iranian ecotypes [26]. In fact, ecotypic differences in the fatty acid profile were present for most of the fatty acids, which accounted for near the 90% of the total fatty acid content. RAK, with the lower biomass production and seed yield of the two ecotypes, exhibited higher contents of the polyunsaturated n6c linolenic and saturated stearic acids, and lower contents of the unsaturated n9c oleic and polyunsaturated alpha linolenic acids, but overall there were no clear differences in the total proportion of unsaturated fatty acids.

Concerning the effect of increasing salinity on the fatty acid composition, Reiahisamani et al. [26] reported a small, albeit significant, increase in the relative content of oleic, palmitic and stearic acids, while the relative contents of linoleic and linolenic acids decreased. In addition, there was no interaction between the salinity level and the ecotype. By contrast, in our study salinity did not affect the relative content of the major and most of the minor fatty acids, and a significant ecotype by salinity interaction was only found for alpha linolenic acid. Therefore, our results suggest that while the effect of irrigation salinity on the pattern of fatty acids is negligible, ecotype differences exist.

Total unsaturated acids in our study accounted for around 85% of the total fatty acids, and were therefore somewhat higher than the 75% reported by Roshandel and Shamsi [13] and close to the 90% level stated by Reiahisamani et al. [26]. In our results the two essential unsaturated fatty acids (linoleic and oleic acids) contributed to 80% of the total, which is close to the 85% reported by Reiahisamani et al. [26], whereas in other studies these two fatty acids only represented 15% of the total [13]. Concerning polyunsaturated fatty acids, which are important components of the human diet, it has been reported that they represent about 80% of the total fatty acid profile in *S. europaea*, with n6c linoleic accounting alone for 65% of the seed oil [26], while other studies have not identified linoleic acid but instead have reported a significant proportion (around 10%) of α-linolenic acid [13]. In our study, the two main polyunsaturated fatty acids (n 6c linoleic acid and alpha linolenic) together accounted for about 65 % of the total fatty acids. Therefore, strong differences in the fatty acid composition have been reported for the seeds of this species, even when comparing ecotypes from the same region (UAE versus Iran). Other members of the Salicornia genus are reported to contain high amounts of linoleic acid; around 75% in the case *of S. bigelovii* [61,62] or over 50% for *S. brachiata* [60]. The main saturated fatty acid in *S. bigelovii* was palmitic acid, but its value was nearly three times lower than the 30% level approximated in *S. brachiata* [60]. Our results support the high nutritional quality of *S. europaea* seeds, in terms of protein content and

fatty acid profiles, together with a low sodium content. Moreover, genetic variability in exists for fatty acid composition.

4.6. Seed germination ability

Salinity during growth of parent plants affected the germination of the next generation of seeds. Indeed, the higher the salinity during growth the lower the germination ability, but the negative effect was particularly evident for plants grown under sea water salinity levels. Increasing salinity during germination also negatively affected the rate of germination, but again only when transitioning from brackish water to sea water, whereas hypersaline conditions nearly completely inhibited germination. The inhibitory effect to seed germination of increases in salinity in *S. europaea* has been reported before [63] and is in line with observations in other euhalophyte species of the same genus [30,64]. However, our study also proved that increasing the salinity conditions during growth negatively affected the capacity for germination, particularly when transitioning from brackish water to sea water. It has long been known that *S. europaea* produces dimorphic seeds, with the larger seeds being more salt tolerant than the smaller seeds, and the larger seed have higher germination percentages regardless of the salinity level [31] together with a less intense innate dormancy [32]. In the halophytic *Chenopodium quinoa*, high salinity during parental growth also diminishes the capacity for germination of the next seed generation, and was associated with a reduction in seed size and the volume of the perisperm [65]. In our study the decrease in the TSW as salinity increased (Table 2) suggested a relative increase the proportion of small seeds, even when the decrease in TSW was already evidenced during transitioning from fresh to brackish water. While these results may have implications in terms of agronomical practices when establishing a *S. europaea* in the field, we did not find differences in germination between the two ecotypes.

4.7. Concluding remarks and future research avenues

Besides the specific focus on investigating the existence of phenotypic variability in *S. europaea* ecotypes from the UAE related to growth and seed yield and quality, our study contributes to a better understanding of the physiological response of this species to salinity. This also includes novel aspects such as the effect of growing conditions on the further germination capacity of the seeds produced or the role of K^+ during growth in response to increasing salinity in the irrigation, while the effect of salinity on the quality of the seeds (nitrogen content and fatty acid profile) remain unaffected. Moreover, our study also provides preliminary insights into the mechanisms associated with ecotypic performance in *S. europaea* and the potential tools for its evaluation. In that sense, performance, as reflected by the phenotypic traits of low $\delta^{13}C$ and Na^+ in shoots and seeds and high K^+ and $\delta^{15}N$ in seeds, requires further exploration, and particularly by enlarging the number of ecotypes under consideration. Future research needs to exploit the synergies between physiological approaches and the capacities of omics to examine ecotypic variability and the metabolic response of *S. europaea* to salinity. For example, random amplified polymorphic DNA analysis, as a quick method for detecting genetic variation patterns, together with analysis of RGB images, as means to phenotype plant response to salinity, have been reported recently in *S. europaea*, but as in our study, only two populations were compared [66].

Despite the interest in using *S. europaea* as a crop in extreme saline habitats, a deeper knowledge of the particular metabolic mechanisms conferring the halophytic characteristics of this species may provide strategies for improving plant performance under saline conditions through molecular breeding. Thus, unigene functional annotation analysis has allowed identification of hundreds of ion transporters in this species related to homeostasis and osmotic adaptation as well as a variety of proteins related to cation, amino acid, lipid and sugar transport [67]. These results suggest that a multiplicity of mechanisms may contribute to the halophyte characteristics of this species. For example, specific genes involved in processes such as osmotic adjustment, energy metabolism and photosynthesis have been identified [68], and these may play an important role in acclimation of this species to season-dependent changes in salinity. Another recent study on *S. europaea* concluded that the gene encoding phosphatidylserine synthase participates in plant salt tolerance by regulating phosphatidylserine levels, and hence plasma membrane potential and permeability, which help maintain ion homeostasis [69]. Another example comes from the fact that NH_4^+ detoxification has been argued as an important trait under high salinity that may differentiate halophytes from glycophytes. Indeed, Ma et al. [70] have proposed a model for NH_4^+ detoxification in *S. europaea* in response to salinity.

Besides the adequacy of the endogenous metabolic mechanisms of *S. europea* in managing saline environments, its associated microbiota would also help with adaptation to adverse conditions. *S. europaea* should support a unique assemblage of halotolerant bacterial and fungal endophytes that would be determined by the type and level of salinity at a particular site, and these would also protect the host plant under saline conditions [71]. In fact, the salinity in the root zone soil is crucial in structuring the endophytic community, with halophilic bacteria dominating and potentially involved in the mitigation of salt stress [72]. However, little is known about the role played by associated endophytic bacteria in increasing tolerance of the host plant to nutrient deficiency [72,73]. A better understanding of this domain may provide insights into new species of diazotrophs associated with halophytes, and these may be used to optimize strategies for selecting biostimulants useful in saline soils [73].

Conflict of Interest

I declare, in behalf of all the authors that there is no any conflict of interest concerning the study:

Author contributions

M.S. and J.L.A. conceived and designed the experiment. M.S. and S.T. conducted the greenhouse cultivation, ground measurements and germination studies. F.Z.R and M.D.S. run the lab analyses. F.Z.R. did the statistical analysis under the supervision of J.L.A. and M.D.S. J.L.A. wrote the draft manuscript and M.S., I.A.E, J.B and and M.D.S. revised the manuscript.

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Table 1: Electric conductivity (EC), pH level, and concentrations of chloride (Cl^-), carbon trioxide (CO_3^{2-}), bicarbonate (HCO_3^-), sulfate ($(SO_4)^{2-}$), sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}) in the supplied irrigation water (fresh, brackish and sea). Values are expressed per dS m^{-1} (EC) or per meq l^{-1} (ion concentrations).

Irrigation water	EC (dS m^{-1})	pH	**Ion concentrations**							
			Cl^- (meq l-	CO_3^{2-} (meq l-	HCO_3^- (meq l-	$(SO_4)^{2-}$ (meq l-	Na^+ (meq l-	K^+ (meq l-	Mg^{2+} (meq l-	Ca^{2+} (meq l-
Fresh water	0.3	7.87	3.0	0.00	1.32	0.0	2.0	0.06	0.6	1.6
Brackish water	25.4	7.31	243.0	0.12	3.52	56.2	229.0	3.97	51.0	33.4
Sea water	40.9	7.80	552.0	0.00	3.27	56.5	554.0	11.79	107.0	20.6

Table 2. Effect of different levels of salinity during growth on seed weight, total shoot biomass, number of branches, number of inflorescences per plant, thousand seed weight (TSW) and plant height of two different Salicornia ecotypes (RAK and UAQ) from the UAE. Values are means ± SE of five replicates.

		Yield components					
Ecotype	Salinity (dS m^{-1})	Seed weight $plant^{-1}$ (g)	TSW (g)	Shoot biomass $plant^{-1}$ (g)	Plant height (cm)	Branches $plant^{-1}$	Inflorescences $plant^{-1}$
RAK	0.3	5.58 ± 0.09	0.25 ± 0.01	134 ± 4	50.5 ± 0.5	19 ± 1	429 ± 11
	25	7.32 ± 0.92	0.24 ± 0.01	231 ± 15	66.4 ± 1.3	28 ± 1	518 ± 35
	40	5.34 ± 0.38	0.24 ± 0.00	193 ± 9	56.7 ± 0.6	24 ± 1	480 ± 36
UAQ	0.3	6.46 ± 0.30	0.24 ± 0.01	141 ± 3	55.3 ± 0.5	21 ± 1	465 ± 21
	25	9.74 ± 0.19	0.21 ± 0.01	295 ± 18	81.1 ± 1.0	31 ± 1	615 ± 31
	40	7.92 ± 0.10	0.22 ± 0.00	253 ± 28	64.1 ± 0.7	27 ± 1	535 ± 19
ANOVA							
Ecotype (E)		<0.001	<0.001	0.005	<0.001	<0.001	0.017
Salinity (S)		<0.001	<0.001	<0.001	<0.001	<0.001	0.002
Interaction (S*E)		ns	0.011	ns	<0.001	ns	ns

Table 3. Effect of different levels of salinity during growth on the total nitrogen (N) and carbon (C) contents and the stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope compositions in the shoots and seeds of two different Salicornia ecotypes (RAK and UAQ) from the UAE. Values are means ± SE of five replicates.

		Elemental analysis & stable isotopes							
Ecotype	Salinity (dS m^{-1})	N_{shoot} (%)	C_{shoot} (%)	$\delta^{15}N_{shoot}$ (‰)	$\delta^{13}C_{shoot}$ (‰)	N_{seed} (%)	C_{seed} (%)	$\delta^{15}N_{seed}$ (‰)	$\delta^{13}C_{seed}$ (‰)
RAK	0.3	0.58 ± 0.06	33.4 ± 0.4	4.3 ± 0.2	-33.3 ± 0.1	7.38 ± 0.18	46.6 ± 0.7	8.8 ± 0.3	-30.6 ± 0.1
	25	0.42 ± 0.02	29.2 ± 0.4	6.5 ± 0.5	-32.2 ± 0.2	7.05 ± 0.19	45.3 ± 0.2	11.3 ± 0.3	-29.5 ± 0.2
	40	0.41 ± 0.01	25.3 ± 0.8	8.9 ± 0.5	-31.4 ± 0.1	7.56 ± 0.18	44.4 ± 0.3	12.7 ± 0.2	-29.1 ± 0.3
UAQ	0.3	0.51 ± 0.02	34.3 ± 0.4	5.3 ± 0.1	-33.6 ± 0.1	6.04 ± 0.19	47.0 ± 0.3	8.1 ± 0.3	-32.4 ± 0.1
	25	0.40 ± 0.02	29.7 ± 0.4	7.8 ± 0.2	-32.6 ± 0.1	6.31 ± 0.25	46.9 ± 0.5	11.4 ± 0.2	-30.7 ± 0.1
	40	0.44 ± 0.02	27.7 ± 1.1	8.7 ± 0.3	-31.8 ± 0.2	6.17 ± 0.13	46.3 ± 0.2	11.9 ± 0.2	-30.4 ± 0.3
ANOVA									
Ecotype (E)		ns	0.025	0.042	0.011	<0.001	0.002	ns	<0.001
Salinity (S)		<0.001	<0.001	<0.001	<0.001	ns	0.015	<0.001	<0.001
Interaction (S*E)		ns	ns	Ns	ns	ns	ns	ns	ns

Table 4. Effect of different levels of salinity during growth on the ion concentration of shoots and seeds of two different Salicornia ecotypes (RAK and UAQ) from the UAE. Values are means ± SE of five replicates.

		Ion concentration (mmol g^{-1} dry matter) and sodium/potassium ratios											
Ecotype	Salinity (dS m^{-1})	Na^+_{shoot}	K^+_{shoot}	Na^+/K^+_{shoot}	P_{shoot}	Ca^{2+}_{shoot}	Mg^{2+}_{shoot}	Na^+_{seed}	K^+_{seed}	Na^+/K^+_{seed}	P_{seed}	Ca^{2+}_{seed}	Mg^{2+}_{seed}
RAK	0.3	3.67 ± 0.14	0.26 ± 0.03	15.56± 2.43	0.11 ± 0.01	0.22 ± 0.02	0.25 ± 0.02	0.55 ± 0.03	0.22 ± 0.01	2.47±0.12	0.32 ± 0.01	0.06 ± 0.01	0.16 ± 0.01
	25	4.96 ± 0.05	0.30 ± 0.02	17.11± 1.02	0.06 ± 0.01	0.28 ± 0.01	0.34 ± 0.02	1.31 ± 0.15	0.26 ± 0.01	5.06±0.66	0.28 ± 0.01	0.06 ± 0.01	0.15 ± 0.01
	40	5.86 ± 0.15	0.37 ± 0.01	15.79± 0.78	0.08 ± 0.01	0.22 ± 0.01	0.39 ± 0.01	1.71 ± 0.25	0.27 ± 0.01	6.47±1.00	0.27 ± 0.01	0.06 ± 0.01	0.15 ± 0.01
UAQ	0.3	3.46 ± 0.13	0.37 ± 0.01	9.52±0.62	0.07 ± 0.01	0.20 ± 0.01	0.23 ± 0.01	0.70 ± 0.03	0.31 ± 0.01	2.23±0.08	0.28 ± 0.01	0.05 ± 0.01	0.15 ± 0.01
	25	4.36 ± 0.15	0.47 ± 0.05	9.80±0.98	0.05 ± 0.01	0.23 ± 0.01	0.32 ± 0.01	1.28 ± 0.10	0.32 ± 0.01	3.97±0.29	0.26 ± 0.01	0.06 ± 0.01	0.15 ± 0.01
	40	4.70 ± 0.31	0.64 ± 0.02	7.35±0.47	0.06 ± 0.01	0.21 ± 0.02	0.40 ± 0.02	1.43 ± 0.10	0.32 ± 0.01	4.55±0.43	0.25 ± 0.01	0.05 ± 0.01	0.14 ± 0.01
ANOVA													
Ecotype (E)		<0.001	<0.001	<0.001	0.003	0.016	ns	ns	<0.001	0.037	<0.001	ns	0.046
Salinity (S)		<0.001	<0.001	ns	0.001	0.008	<0.001	<0.001	0.001	<0.001	<0.001	ns	ns
Interaction (S*E)		Ns	0.046	ns	ns	ns	ns	ns	0.009	ns	ns	ns	ns

Table 5. Regression coefficients of the Pearson correlation between different physiological traits and branches plant^{-1}. For each correlation five replicates of the two ecotypes within a given salinity level were used.

	Ion concentration									
	Na^{+}_{shoot}	K^{+}_{shoot}	P_{shoot}	Ca^{2+}_{shoot}	Mg^{2+}_{shoot}	Na^{+}_{seed}	K^{+}_{seed}	P_{seed}	Ca^{2+}_{seed}	Mg^{2+}_{seed}
0.3 dS m^{-1}	ns	0.635*	ns	ns	ns	0.677*	ns	ns	ns	ns
25 dS m^{-1}	-0.851**	ns	ns	ns	ns	ns	ns	ns	ns	ns
40 dS m^{-1}	-0.808*	0.853**	ns	ns	ns	ns	0.828**	ns	-0.746*	-0.763*

	Stable isotope composition and elemental analysis							
	N_{shoot}	C_{shoot}	$\delta^{15}N_{shoot}$	$\delta^{13}C_{shoot}$	N_{seed}	C_{seed}	$\delta^{15}N_{seed}$	$\delta^{13}C_{seed}$
0.3 dS m^{-1}	ns	ns	0.703*	ns	-0.779**	ns	ns	-0.728*
25 dS m^{-1}	ns	ns	ns	ns	-0.798**	ns	ns	-0.638*
40 dS m^{-1}	ns	ns	ns	ns	-0.814**	0.683*	ns	ns

Table 6. Effect of different levels of salinity during growth on the relative contents of fatty acids and the total lipid content of seeds of two different Salicornia ecotypes from the UAE. Values are means ± SE of five replicates.

Fatty acids (FA) (% of total FA dry seed)	RAK			UAQ			ANOVA		
	0.3 (dS/m)	25 (dS/m)	40 (dS/m)	0.3 (dS/m)	25 (dS/m)	40 (dS/m)	Genotype (G)	Salinity (S)	Interaction (S*G)
14:0 miristic acid	0.11 ± 0.01	0.11 ± 0.00	0.15 ± 0.02	0.13 ± 0.01	0.14 ± 0.01	0.12 ± 0.00	ns	ns	ns
15:0 pentadecanoic acid	0.05 ± 0.01	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.00	0.030	ns	ns
16:0 palmitic acid	11.56 ± 0.90	10.86 ± 0.27	12.21 ± 0.55	10.62 ± 0.22	11.03 ± 0.28	11.10 ± 0.34	ns	ns	ns
16:1 palmitoleic acid	0.10 ± 0.01	0.11 ± 0.00	0.15 ± 0.02	0.16 ± 0.02	0.17 ± 0.01	0.14 ± 0.00	0.004	ns	0.025
16:1 (unidentified)	0.10 ± 0.01	0.10 ± 0.01	0.15 ± 0.02	0.12 ± 0.02	0.12 ± 0.01	0.11 ± 0.01	ns	ns	ns
17:0 margaric acid	0.14 ± 0.00	0.13 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.11 ± 0.00	<0.001	0.004	ns
17:1 cis-10-heptadecenoic acid	0.18 ± 0.02	0.18 ± 0.01	0.23 ± 0.02	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.00	0.041	ns	ns
18:0 stearic acid	2.70 ± 0.30	2.52 ± 0.09	2.62 ± 0.12	1.72 ± 0.22	1.56 ± 0.31	2.34 ± 0.08	0.001	ns	ns
18:1 n9c oleic acid	18.86 ± 1.30	16.83 ± 0.24	17.09 ± 0.69	19.86 ± 0.74	19.55 ± 0.92	21.97 ± 0.66	0.001	ns	ns

18:1 (unidentified)	0.64 ± 0.06	0.64 ± 0.02	0.76 ± 0.06	0.86 ± 0.02	0.91 ± 0.03	0.92 ± 0.01	<0.001	ns	ns
18:2 n6c linoleic acid	60.78 ± 3.07	63.53 ± 0.68	61.33 ± 1.42	59.44 ± 0.93	58.32 ± 1.31	56.13 ± 1.23	0.015	ns	ns
18:3 alpha linolenic	3.62 ± 0.29	3.74 ± 0.08	4.33 ± 0.18	4.99 ± 0.21	6.02 ± 0.44	4.66 ± 0.15	<0.001	ns	0.008
20:0 araquidic acid	0.31 ± 0.04	0.28 ± 0.03	0.32 ± 0.02	0.26 ± 0.02	0.30 ± 0.06	0.40 ± 0.02	ns	ns	ns
20:1 n9 cis 11-eicosenoic acid	0.25 ± 0.03	0.22 ± 0.01	0.24 ± 0.02	0.37 ± 0.04	0.36 ± 0.06	0.48 ± 0.01	<0.001	ns	ns
20:2 cis-11,14-eicosadienoic acid	0.07 ± 0.01	0.07 ± 0.00	0.07 ± 0.01	0.09 ± 0.00	0.09 ± 0.01	0.10 ± 0.00	<0.001	ns	ns
22:0 behenic acid	0.10 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.037	ns	ns
22:1 Erucic acid	0.08 ± 0.01	0.10 ± 0.03	0.05 ± 0.02	0.09 ± 0.00	0.13 ± 0.02	0.10 ± 0.00	0.049	0.029	ns
24:0 lignoceric acid	0.13 ± 0.02	0.14 ± 0.00	0.15 ± 0.03	0.15 ± 0.04	0.15 ± 0.03	0.15 ± 0.01	ns	ns	ns
24:1 nervonic acid	0.07 ± 0.02	0.05 ± 0.02	0.10 ± 0.00	0.09 ± 0.01	0.06 ± 0.03	0.10 ± 0.00	ns	ns	ns
polyunsaturated FA	0.10 ± 0.02	0.10 ± 0.01	0.12 ± 0.01	0.14 ± 0.02	0.15 ± 0.01	0.16 ± 0.00	0.013	<0.001	ns
Total lipid extract (% seed dry matter)	26.58 ± 3.31	35.47 ± 0.98	32.93 ± 1.31	32.47 ± 0.41	34.76 ± 0.81	33.22 ± 0.51	ns	0.013	ns

Table 7. Effect of different levels of salinity applied during growth: fresh water (FW, 0.3 dS m^{-1}), brackish water (BW, 25 dS m^{-1}), sea water (SW, 40 dS m^{-1}) and during germination: FW, BW, SW and hypersaline water (HW, 80 dS m^{-1}), on the germination rate of two different Salicornia ecotypes (UAQ and RAK) from the UAE. Values are means ± SE of 3 replicates.

Ecotype	Salinity growth	Salinity germination	% of germination	Ecotype	Salinity Growth	Salinity germination	% of germination
UAQ	FW	FW	74.33 ± 3.72	RAK	FW	FW	70.67 ± 4.11
		BW	64.00 ± 4.23			BW	76.00 ± 3.10
		SW	37.00 ± 3.08			SW	25.00 ± 6.54
		HSW	0			HW	1.67 ± 0.26
	BW	FW	60.67 ± 398		BW	FW	66.67 ± 2.82
		BW	62.33 ± 1.41			BW	62.33 ± 3.59
		SW	37.67 ± 3.34			SW	30.00 ± 0.77
		HSW	1.00 ± 0.00			HW	1.00 ± 0.38
	SW	FW	4.00 ± 0.38		SW	FW	56.33 ± 2.44
		BW	53.67 ± 1.80			BW	55.33 ± 2.18
		SW	21.33 ± 4.36			SW	34.00 ± 0.38
		HW	1.33 ± 0.26			HW	4.67 ± 0.64

ANOVA	
Ecotype	ns
Salinity growth	<0.001
Salinity germination	<0.001
Ecotype*Salinity during growth	ns (P=0.059)
Ecotype*Salinity during germination	ns
Salinity during growth*Salinity during germination	0.001
Ecotype*Salinity during growth*Salinity during germination	ns (P=0.055)

Figure 1. Left: geographical location and characteristics of the two Salicornia ecotypes collected in the UAE: Ra's al Khaimah (RAK) and Umm al-Qaiwain (UAQ). The UAQ ecotype starts flowering and maturing one week earlier than the RAK ecotype. The spikes of RAK turn yellow before maturing, while those of UAQ turn pink during the same stage (Shahid 2017). Right: plants of both ecotypes growing in pots within a greenhouse of the ICBA Experimental Field (Dubai).

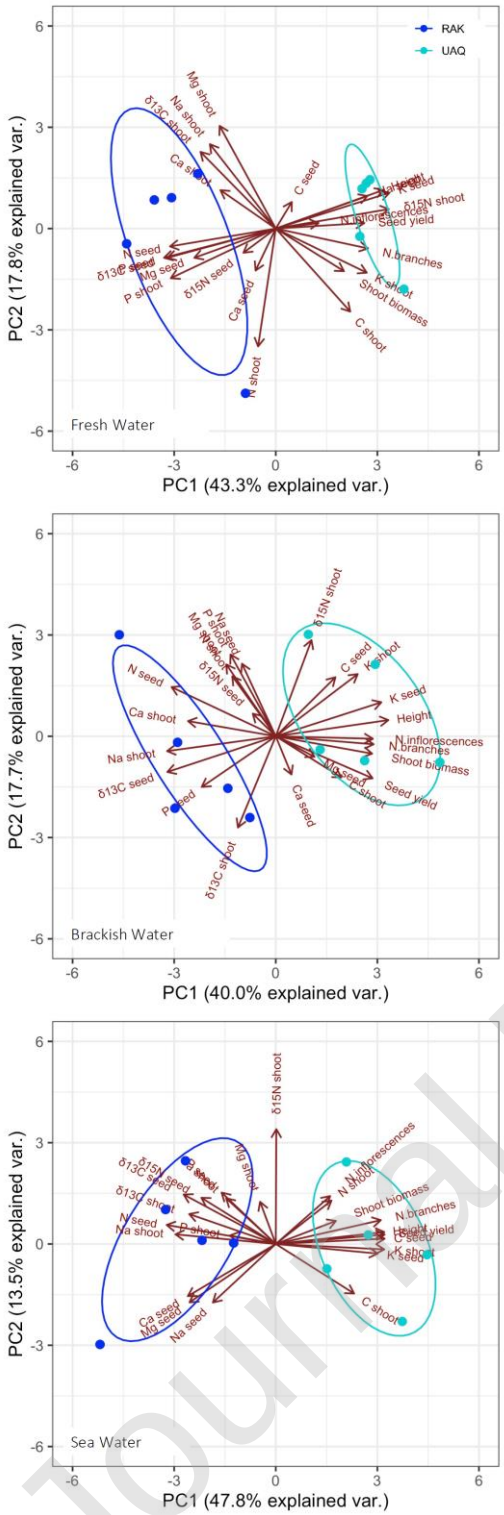

Figure 2. Principal component analysis (PCA) of the two *S. europaea* ecotypes growing under any of the three different irrigation conditions assayed (fresh water,; brackish water and sea water), using as variables the seed yield per plant (Seed yield), plant dry biomass (Shoot Biomass), plant height (Height), number of inflorescences per plant (N. inflorescences), number of branches per plant (N branches), the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope compositions and the carbon (C) and nitrogen (N), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and phosphorus contents in the seeds and

shoots. Red and blue colouring represents samples from each of the two ecotypes (RAK and UAQ).

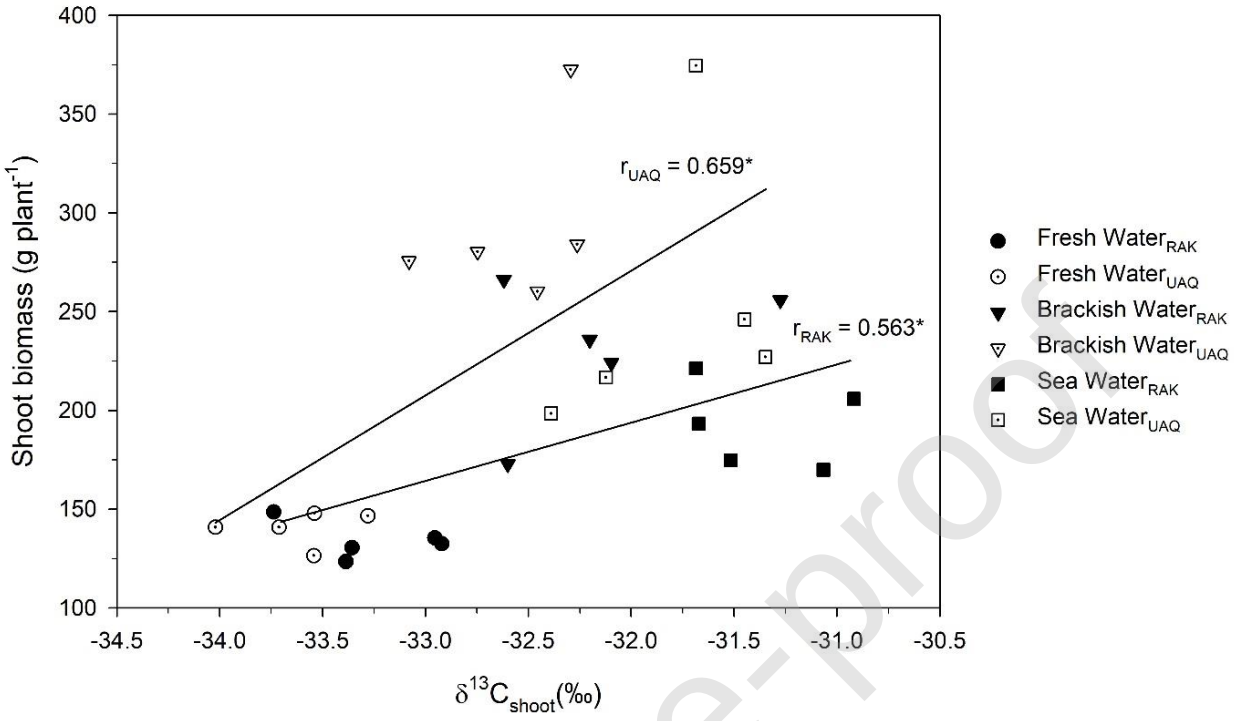

Figure 3. Relationship between carbon isotope composition ($\delta^{13}C$) of the shoot biomass for each ecotype (UAQ and RAK) across the three irrigation conditions (fresh, brackish and sea water).

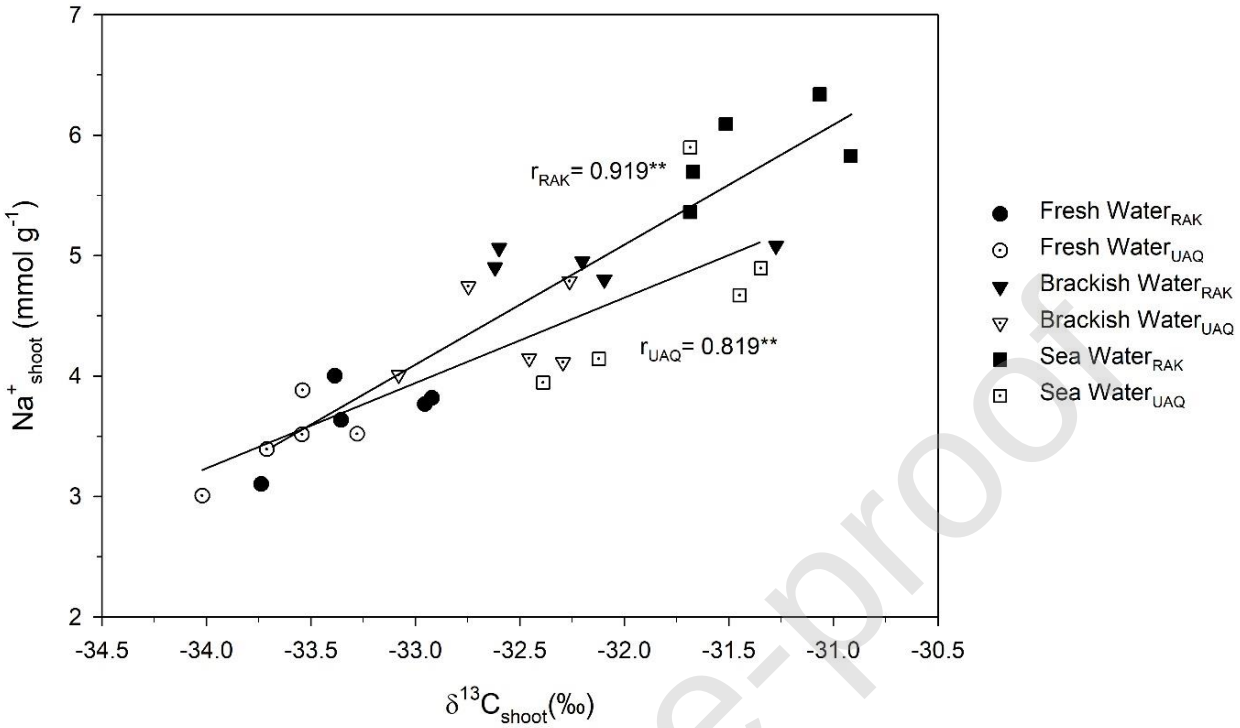

Figure 4. Relationship between carbon isotope composition ($\delta^{13}C$) of the shoot and Na^+ content in the shoots of each ecotype (UAQ and RAK) across the three irrigation conditions (fresh, brackish and sea water).

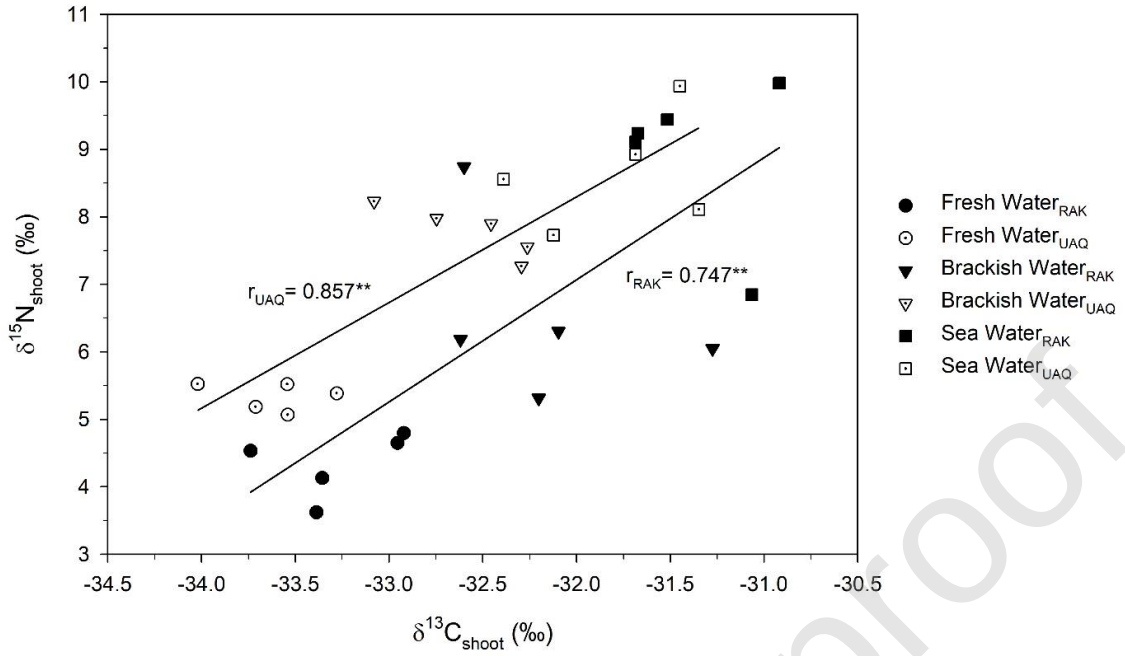

Figure 5. Relationship between carbon isotope composition ($\delta^{13}C$) and nitrogen isotope composition ($\delta^{15}N$) of the shoots for each ecotype (UAQ and RAK) across the three irrigation conditions (fresh, brackish and sea water).

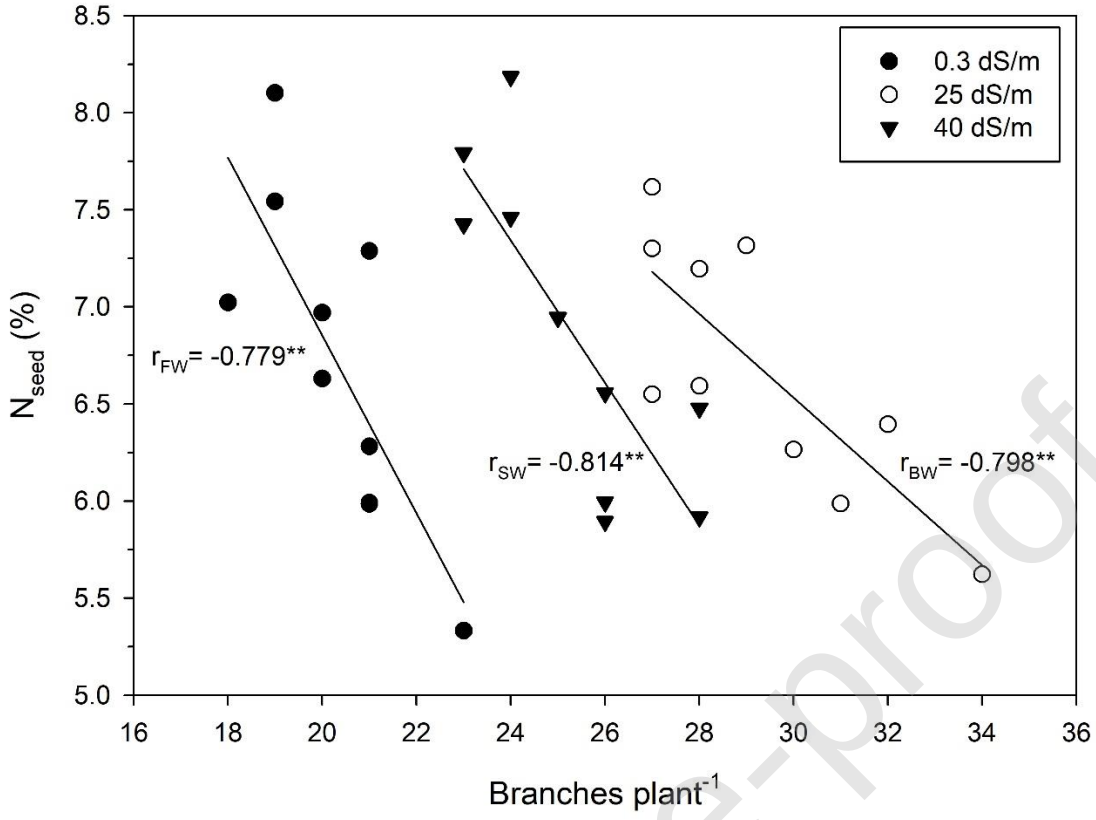

Figure 6. Correlations between the nitrogen content of the seeds and the number of branches in Salicornia for each salinity level.