

Successful Cultivation of *Salicornia brachiata* – A Sea Asparagus Utilizing RO Reject Water: A Sustainable Solution

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Abstract

Reverse Osmosis (RO) is an effective technique to get potable water from brackish water. However, disposal of the high TDS reject RO water is a concern. The conventional methods used for disposal of RO reject water are expensive and not environmental friendly. Herein, the present study is an attempt to grow *Salicornia brachiata* using RO reject water; this approach provides a sustainable solution for utilizing RO reject water. The optimum plant growth was observed when irrigated with RO reject water (A-type) having TDS of 26511-27102 ppm. Additionally, the biomass of plants was found moderately better when treated with A-type reject water compared to the plants irrigated with high saline water (sea water) having TDS of similar range i.e., 27511-28010 ppm TDS. The highest values of succulence (phyloclade diameter) coincide with the growth optimum. The optimum inflorescence length was noted in A-type RO reject water and sea water treated plants. The moisture content in the plants was insignificantly different at different TDS. On the other hand, the short height plants were developed with less number of branches and biomass, when treated with A-type RO reject water having F concentration of 25 and 50 mg/L. The test results of phyloclade for F- was found in the range of 0.09-0.12 mg/100 gm DW indicates that *S. brachiata* is F- tolerant plant. Therefore, the finding suggest that cultivating *S. brachiata* plant as a vegetable in greenhouse using RO reject water with and without F- is potential as well as environmental friendly solution for reject water management.

Keywords: Brackish; Cultivation; Fluoride; RO reject water management; *Salicornia brachiata*

Introduction

Increasing underground water TDS has intensifying demand of potable water, which led to several technologies in water and wastewater treatment. Both brackish (>5% sodium chloride) and high F- containing water cannot be used for drinking/irrigation purpose. The common F-bearing minerals are fluorospar (CaF₂), cryolite (Na₃AlF₆), and chiolite (Na₃Al₃F₁₄) are present in the soil (WHO, 1984). NaF, KF and NH₄F are readily soluble in the acid soil [1] and leach into ground water. The presence of these aforesaid mentioned contaminants in the ground water has extended the use of desalination technology to get potable water.

The customized membrane based desalination plants area available as per the quality of water which as a consequence generates huge amount of permeates as reject water. Essentially, RO membranes allow (30 Da–0.001 μ) partial water to pass through while rejecting the rest water (saline effluent from desalination plants) is normally viewed as a severe environmental threat. Several disposal techniques of the brine concentrate are practiced worldwide as disposal of reject water is considered as a major challenge. These include direct surface water discharge, discharge to a sewage treatment plant, deep well disposal, evaporation ponds, brine concentrators, and mixing with the cooling water or sewage treatment effluents before surface discharge [2]. However, these available options may deem infeasible due to the various reasons.

The utilization of reject brackish RO reject water for the irrigation of halophytes could be a sustainable and environmental friendly approach. The Halophytes are salt-resistant or salt-tolerant plants which can grow in moderate to high saline soil by utilizing salinity for their growth [3]. Halophytes can be obligatory halophytes, preferential halophytes, supporting halophytes, accidental halophytes [4], obligatory halophyte (*Salicornia brachiata*) require salinity for survival as well as optimum growth. The halophyte *Salicornia brachiata* Roxb belongs to the Amaranthaceae family. This halophyte

has a broad geographical distribution, i.e., widely available in Eurasia, North America and South Africa [5] whereas well distributed in Indian coastal area of Tamil Nadu, Andhra Pradesh, Orissa, Gujarat, and Bengal. Apart from culinary relevance, *Salicornia* attributes its medicinal importance in various diseases such as immunomodulatory, lipid-lowering, anti-proliferative, osteoprotective, and hypoglycemic, anti-oxidative stress, inflammation, diabetes, asthma, hepatitis, cancer, gastroenteritis. Presence of selenium, an essential micronutrient for growth and robust antioxidant effects [6]. *Salicornia spp.* is rich in vitamins, minerals, amino acids [7]. Hence, it can be exploited for the treatment of various free radical mediated ailments [8]. *Salicornia* has also been used as a source of soda (sodium carbonate) in glass making industries [9]. *S. brachiata* seed are rich in sulfur amino acids and 55–64% polyunsaturated fatty acids [6]. *S. herbacea* seed oil can be used in food processing [10]. Optimum *Salicornia* biomass was achieved at 25 dS/m salinity level (www.biosaline.org). The plant is reported to have stress tolerant genes [11,12]. These genes help plant to withstand in stressed condition. The optimum relative growth rates of *Salicornia dolichostachya* was achieved at 500 mM salinity in hydroponic culture in a greenhouse [13]. *Salicornia* is not just highly salt tolerant plant but the optimum plant growth is stimulated at 150-300 mM NaCl. Good amount of harvestable biomass of *Salicornia ramosissima* could be generated using artificial sea water containing 257 mM NaCl [14],

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plant growth was depressed at low salinity [15]. *Salicornia* spp. have been grown in aquaculture systems and can be used for edible purpose [16]. Due to the presence of rhizobacteria in its roots, its cultivation is may be beneficial for increasing soil fertility and phyto-remediation of bromide [17]. *Salicornia* accumulates good amount of salt in its tissues, hence, it has been used to prepare vegetable salt “Saloni” by Council of Industrial Research-Central Salt and Marine Chemicals Research Institute (CSIR-CSMCRI), Bhavnagar, Gujarat, and recommended for high blood pressure patients due to low sodium content [18,19]. Culinary, medicinal and industrial uses of this plant make it a unique halophyte for commercial application.

The conventional disposal/remediation methods may cost anywhere from US \$10 - 1000 per cubic meter [20]. Hence, the use of reject water for the cultivation of the salinity tolerant plants open doors for effective utilization of reject water. Due to the high salinity tolerance, higher growth rate, short gestation period, producing soybean quality oil seeds and agronomic potential, *Salicornia* spp. can become important plant for the cultivation [21] using RO reject water. *Salicornia* is grown in several parts of the world [22]. *S. brachiata* being halophyte require salinity for its optimum growth, hence, cultivation of *Salicornia* in greenhouse will require irrigation with saline water (NaCl) for optimum growth.

The cultivation of *Salicornia brachiata* using brackish RO reject water has not been reported till today which is a sustainable as well as environmental friendly option. Thus, the present study for the first time focuses on the utilization of RO rejected water for cultivating *S. brachiata* plant as a vegetable in greenhouse which is potential as well as environmental friendly solution for reject water management. The approach also provides the greenhouse cultivation method of *Salicornia brachiata* without using any additional nutrients.

Materials and Methods

RO reject water treatment

Two months old seedlings were transplanted in garden soil and farm yard manure (FYM) mix (3:1). Plants adapted to greenhouse conditions were treated with RO rejected water collected from CSMCRI RO units, reject water TDS was ranging 5213-5316 ppm. NaCl was added to RO reject water in different concentration, (A-type) 15 gm/L NaCl + RO rejected water, the final TDS of water was ranging 27511-28010 ppm TDS, (B-type) RO rejected water +10 gm NaCl and the final TDS was ranging 12102-12521 ppm and, (C-type) RO reject water ranging 5213-5316 ppm. Plants treated with sea (S) water (27501-28006 ppm TDS) were used as positive control to compare the growth with rejected treated plants. Plants treated with tape (T) water (391-415 ppm) were used as negative control. The greenhouse conditions were 18-h light/6-h dark by natural sunlight, photosynthetically active radiation at the canopy level averaging $397 \pm 34 \mu \text{mol m}^{-2} \text{s}^{-1}$ and 35-40°C light/28-30°C dark with a relative humidity between 50- 60%.

Fluoride treatment

A stock solution was prepared by dissolving 221 mg NaF in 1.00 L of deionized water (100 mg F/L). Appropriate dilutions were made to give 25 and 50 mg F ion/L. Plants adapted to greenhouse conditions were irrigated with RO rejected water (A-type) containing 25 and 50 mg F ion /L. Control was considered as plants grown under the same conditions and irrigated with RO reject water (type-A) without NaF.

Measurement of plant growth and biomass

Shoot girth of six month old plants was measured by Vernier

calliper of all treatments. Same age plants were measured for canopy, plant height, fresh weight (FW), dry weight (DW), spike (inflorescence) and canopy width. Plants were uprooted and fresh weight was note, these plants were dried at 70°C in oven for 7 days and dry weight was noted.

Analyses of Na⁺, Ca²⁺, K⁺, Mg²⁺, Cl⁻, F⁻

All water samples were analysed for Na⁺, K⁺, Mg²⁺, Ca²⁺ by Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and Cl⁻ and F⁻ content were analysed by Thermos Scientific Dionex ICS-5000+DC.

Results and Discussion

Water is the most valuable natural resource for life and the demand on potable water is growing steadily and is becoming one of the worldwide challenges. The cause of the upsurge in salt concentration is due to overuse of groundwater for different purpose, which led to increase in ground water salinity and simultaneous shortage of drinking water. Therefore scarcity of water for drinking purpose has led to the technological advancement in applications of membrane processes for potable water. Though RO is advanced technology to get potable water by desalination of brackish water, however, utilization of reject water is a great challenge. Thus, present work is an attempt for the utilization of RO reject water for the cultivation purpose. To best of our knowledge, we are the first to report such studies. High TDS of reject water is the main limiting factor for its use in agriculture.

Effect of RO reject water on plant growth

It was determined by the water analysis that major elements Ca and Mg content were higher in A-type RO reject water compared to sea water. K, Na, and Cl content of A-type water was moderately similar to sea water (Table 1). The plant growth was very poor in low TDS water (Figure 1A). As TDS of irrigation water increases the plant growth and development also increases. Optimum growth was observed in A-type RO reject water (Figure 1B). Ca and Mg content in A-type reject water were higher compared to positive control but no adverse effect on plant growth was noted. Singh et al. [14] reported the presence of ascorbate peroxidase (APX) that makes this plant tolerant to oxidative stress and thus confers abiotic stress tolerance. Maximum plant height (42.3 ± 1.7 cm), length of inflorescences (7.6 ± 0.6 cm) was observed in A-type reject water irrigated plants and $98.3 \pm 7.4\%$ shoots possessed inflorescence/spikes (Table 2). However, plant height, number of branches, length of inflorescences and shoots possessed inflorescence were insignificantly different with positive control and significantly different with negative control and C-type RO reject water treated plants. Growth and development of the plants were significantly different at increasing TDS of water. The highest fresh weight (321.2 ± 8.5 gm) was noted in plants treated with A-type reject water (Table 3). Glycophytes salinity tolerances limit range between 50-250 mM [23-25] and salinity beyond this limit is toxic to the plants. *Salicornia* is one among the halophytes that has sustainability to grow in high saline land. Fresh and dry weight of *S. rubra* increased with an increase in salinity. Optimal growth of *S. rubra* plants were recorded at 200 mM NaCl [26]. *S. dolichostachya* had its optimum growth at 300 mM NaCl. *S. bigelovii* is one of the most salt-tolerant plant species that grows normally, in two time's greater seawater salinity [27]. In the present study, A-type reject water was found optimum for the plant growth. FW and DW of *Salicornia dolichostachya* increased when irrigated with 50-300 mM NaCl [13]. *S. brachiata* could successfully grow in field having TDS of 34000 ppm [28]. There was a significant increase in *S. brachiata* plant growth when treated with A-type reject water. In the present study, the

| Samples | Na ⁺ (mg/L) | Cl ⁻ (mg/L) | K ⁺ (mg/L) | Mg ²⁺ (mg/L) | F ⁻ (mg/L) | Ca ²⁺ (mg/L) |
|------------------|------------------------|------------------------|-----------------------|-------------------------|-----------------------|-------------------------|
| Tape water (T) | 48 | 0.0 | 0.69 | 35 | 0.0 | 39 |
| A-type RO reject | 6635 | 900 | 125 | 1940 | 10 | 3011 |
| B-type RO reject | 4330 | 570 | 121 | 1938 | 8.5 | 2945 |
| C-type RO reject | 3987 | 482 | 122 | 1931 | 8.0 | 2936 |
| Sea water (S) | 6550 | 970 | 517 | 1440 | 0.0 | 5165 |

Table 1: Water sample analysis for major elements.



Figure 1: Plant treated with tape water (A), plants treated with A-type RO reject water (B), plant treated with sea water (C), uprooted plant treated with A-type RO reject water (D), salinity effect on inflorescence length, tape water (E), B-type RO reject water (F), sea water (G) A-type RO reject water (H), plant treated with A-type RO reject water + 50 ppm F⁻ (I).

| Treatment | Plant height (cm) | No of branches | Length of inflorescence (cm) | Shoots having inflorescence (%) | Total Chlorophyll (mg/kg) |
|------------------------------|--------------------------|-------------------------|------------------------------|---------------------------------|---------------------------|
| Tape water (T) | 19.3 ± 1.1 ^c | 8.2 ± 1.1 ^b | 1.2 ± 0.3 ^e | 13 ± 1.1 ^c | 387 ± 6.4 ^a |
| A-type RO reject | 42.3 ± 1.7 ^a | 38.1 ± 1.6 ^a | 7.6 ± 0.6 ^a | 98.3 ± 7.4 ^a | 99 ± 2.0 ^d |
| B-type RO reject | 31.8 ± 1.5 ^b | 16.3 ± 1.2 ^b | 5.3 ± 0.4 ^c | 61 ± 2.3 ^b | 210 ± 3.6 ^b |
| C-type RO reject | 30.3 ± 1.45 ^b | 10.1 ± 1.0 ^b | 4.6 ± 0.6 ^d | 55 ± 1.7 ^b | 216 ± 8.3 ^b |
| Sea Water (S) | 39.9 ± 0.6 ^a | 39.3 ± 2.0 ^a | 6.0 ± 1.0 ^b | 98.6 ± 10.1 ^a | 103 ± 2.3 ^d |
| A-type+25 ppm F ⁻ | 26.6 ± 0.8 ^b | 31.3 ± 1.2 ^a | 6.3 ± 0.3 ^b | 96.3 ± 2.7 ^a | 122 ± 5.0 ^c |
| A-type+50 ppmF ⁻ | 22.6 ± 1.2 ^b | 32.3 ± 0.8 ^a | 6.3 ± 0.8 ^b | 91.6 ± 2.0 ^a | 109 ± 1.7 ^d |

Note: Data presented as mean ± SE. Means followed by the same letter within columns are not significantly different at 5% probability level

Table 2: Effect of reject water TDS and F⁻ on growth, flowering and chlorophyll of *Salicornia brachiata*.

biomass of the plant treated with all the three types of reject water was greater than tape water. C-type RO reject water (deprive of the salinity) treated plants developed better plant growth compared to negative control plants. This result reveals that RO reject water has no adverse effect on the *S. brachiata* growth and plant growth was better at high TDS. However, optimum growth was observed in A-type RO reject water and Sea water treated plants. It was known by the study that *S. brachiata* is “salt loving plant”.

Salinity induced increase in canopy can be interpreted as adaptive

mechanisms to high salinity. Also the plant biomass increases with increasing TDS of water. No significant difference was noted in plant height, number of branches, fresh weight, length of spike (Figures 1B-1D) and total chlorophyll content of RO rejected water and positive control plants (irrigated with sea water). In the present study, also the chlorophyll content declined with increase in the salinity. Similar effects were observed in *Salicornia prostrate* and *Suaeda prostrate* [29]. The spike length was influenced by water TDS (Figures 1E and 1H), maximum 7.6 ± 0.6 cm spike length was noted in type-A reject water and minimum length was observed in negative control (T) plants

| Treatment | Fresh Weight (gm) | Dry Weight (gm) | Moisture content (%) | Diameter of phylloclade (cm) | Canopy (cm) |
|-------------------------------|--------------------------|-------------------------|-------------------------|------------------------------|-------------------------|
| Tape water (T) | 126.9 ± 3.6 ^d | 19.7 ± 1.7 ^c | 84.4 ± 1.0 ^a | 0.23 ± 0.03 ^c | 24.3 ± 2.8 ^b |
| A-type RO reject | 321.2 ± 8.5 ^a | 45.1 ± 2.6 ^a | 85.9 ± 0.2 ^a | 0.50 ± 0.05 ^b | 37.2 ± 3.7 ^a |
| B-type RO reject | 226.7 ± 3.7 ^b | 30.8 ± 2.6 ^b | 86.4 ± 0.9 ^a | 0.40 ± 0.05 ^b | 30.9 ± 4.5 ^a |
| C-type RO reject | 172.1 ± 2.8 ^c | 25.6 ± 2.3 ^b | 85.1 ± 2.1 ^a | 0.46 ± 0.03 ^b | 29.8 ± 2.4 ^b |
| Sea Water (S) | 313.1 ± 8.6 ^a | 46.3 ± 2.7 ^a | 85.2 ± 1.3 ^a | 0.63 ± 0.03 ^a | 36.1 ± 3.8 ^a |
| A-type +25 ppm F ⁻ | 201.9 ± 7.5 ^b | 28.3 ± 1.7 ^b | 85.9 ± 1.8 ^a | 0.56 ± 0.03 ^b | 31.3 ± 3.2 ^a |
| A-type +50 ppm F ⁻ | 171.8 ± 2.6 ^c | 24.1 ± 1.6 ^b | 85.8 ± 1.0 ^a | 0.50 ± 0.05 ^b | 30.3 ± 3.6 ^a |

Note: Data presented as mean ± SE. Means followed by the same letter within columns are not significantly different at 5% probability level

Table 3: Effect of reject water on fresh, dry, moisture content, phylloclade and canopy of *Salicornia brachiata*.

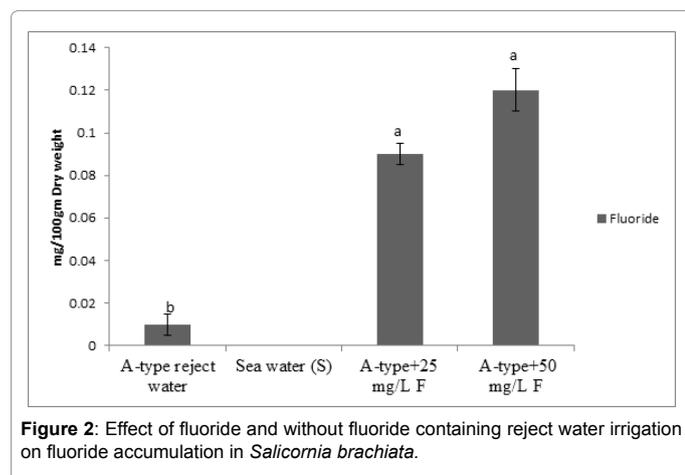


Figure 2: Effect of fluoride and without fluoride containing reject water irrigation on fluoride accumulation in *Salicornia brachiata*.

(Figure 1H). The spike length of positive control (S) and type-A reject water treated plants were insignificantly different. Also, 25-30 day delay in the flowering was noted in negative control plant as compared to A-type reject water treated plants and positive control plants (data not shown). Poor (126.9 gm) fresh weight (FW) was noted in negative control as compared to C-type reject water treated plants. These results revealed that RO reject water can be used for the cultivation of the plant, as optimum 321.2 gm FW was noted in A-type reject water (Table 3). TDS of water has no significant effect of on moisture content of the plants. The succulence of phylloclade of A-type treated plants were thicker (0.50 ± 0.05 cm) than tape water treated plants (Table 3). This shows that RO reject water has no adverse effect on plant growth and development (Figures 1 and 2). It was also known by the study that *Salicornia* plants were tolerant to high calcium and magnesium content and did not have any adverse effect on plant growth. Hence, reject water can be used for the cultivation without compromising with the biomass.

Effect of F⁻ containing RO reject water on plant growth

Plants irrigated with F⁻ containing A-type RO reject water have significant different plant height, fresh weight, length of inflorescence, and total chlorophyll as compared to positive control (Tables 2 and 3). The F⁻ content in A-type reject water treated plants were comparatively low as compared to A-type reject water +50 mg/L F. In the test results of phylloclade for F⁻ treatment were found to possess 0.09-0.12 mg/100 gm fluoride (Figure 2). *Camellia sinensis* (Tea) is known for its tendency to accumulate high fluoride. Waugh et al. [30] reported concentration of fluoride ranged from 1.6 to 6.1 mg/L in tea infusions. The European Food Safety Authority (EFSA) and European Union reported a daily dietary intake by adult of 6.0 mg/day and 7.0 mg/day fluoride per day, respectively (<http://www.efsa.europa.eu>). The concentration of F⁻ in *S. brachiata* (irrigated with fluoride containing A-type water) is found less

than that of in the Tea. The results revealed that the plant is tolerant to fluoride and F⁻ containing RO reject water can be used for the irrigation purpose; however, there was reduction in the biomass of the plant [31,32].

Conclusion

It was known by the study that RO reject water can be used for the successful cultivation of the *S. brachiata* without compromising with the biomass of the plant. The biomass of plants treated with RO reject treated water was moderately better than the positive control and significantly higher than negative control. Huge amount of reject water obtained from the community scale RO system can be utilized for the large scale cultivation of the *S. brachiata* without the addition of any other nutrients.

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