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Seasonal variations in phytodesalination capacity of two perennial halophytes in their natural biotope

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In Soliman sabkha (NE Tunisia), *Tecticornia indica* and *Suaeda fruticosa* tufts were divided into three size classes (small, medium, and big) in which the shoot sodium and potassium contents were determined in July 2007 and February 2008. Shoot dry weights per tuft and per hectare were estimated. Soil samples (20 upper centimeters) were taken from inside and outside the halophyte tufts and analyzed for electrical conductivity ($EC_{1/10}$) and soluble sodium content. We found that these two parameters were significantly lower in the soil from inside the tufts than in the surrounding soil. This effect was more pronounced in winter (February 2008) when $EC_{1/10}$ inside halophyte tufts was 63 to 72% lower than outside. In July 2007, the relevant reduction of $EC_{1/10}$ inside the tufts was 55%. Soluble sodium content was reduced by 70.5% in winter and only 31 to 37% in summer. The ecosystem productivity was about 8.6 tonnes dry weight per hectare (t DW ha^{-1}) with higher sodium than potassium contents (about 0.646 t Na^+ ha^{-1} in summer and 0.752 tonnes Na^+ ha^{-1} in winter). Regardless of the season, *T. indica* exhibited much higher phytodesalination capacity (77.7-94.4% of the whole shoot-removed sodium). For *S. fruticosa*, the decrease in soil salinity was due to roots that released sodium ions from the exchange sites and facilitated their leaching to the deeper horizons. From an ecological point of view, phytodesalination and sodium leaching enhancement are interesting processes since they provide glycophytes with a microhabitat suitable for their development, which maintains the biodiversity within the saline ecosystem.

Key words: desalination, *Suaeda fruticosa*, *Tecticornia indica*, productivity, sodium accumulation, soil properties.

INTRODUCTION

Several methods were established to reclaim salt-affected soils and were grouped into hydraulic, physical, chemical, and biological approaches (Shahid, 2002). The latter involves several techniques among which, phytodesalination refers to the use of Na-hyperaccumulating plants to remove sodium from the soil. This plant-based method is of great importance especially for several developing countries (Rabhi *et al*., 2009), where chemical amendments are getting more and more expensive (Kumar & Abrol, 1984; Ahmad *et al*., 1990). In arid and semi-arid regions, where precipitation is too low to leach salts from the rhizosphere (Shiyab *et al*., 2003), shoot sodium accumulation could reduce soil sodium content and consequently its salinity (Rabhi *et al*., 2009). Several authors (Abdelly *et al*., 1995; Zhao *et al*., 2001; Ravindran *et al*., 2007; Rabhi *et al*., 2009) encourage the use of Na^+ and Cl^- hyperaccumulating plants for soil desalination that was shown to maintain biodiversity in salt-affected ecosystems and to improve their pro-

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ductivity (Abdelly *et al*., 1995).

Soliman sabkha is a salt-affected ecosystem at 35 km north-east of Tunis. Plants growing in this region are subjected, in addition to salinity, to hard seasonal variations from waterlogging and low temperatures during the humid period of the year to drought, heat stress and soil drying during the dry period. In a previous work, we showed that under greenhouse conditions and without leaching, the two perennial shoot-succulent halophytes, *Tecticornia indica* (Willd.) and *Suaeda fruticosa* (Forssk.) (Chenopodiaceae) exhibited a noticeable capacity to desalinize their rhizosphere (Rabhi *et al*., 2009). In the present investigation, we attempted to evaluate the seasonal variations of phytodesalination capacities in plants growing in their natural biotope.

MATERIALS AND METHODS

Description of the experimental site

During the humid period of the year, Soliman sabkha is waterlogged and not easily-accessible, because of the soil structure (23% clay, 55% silt, and 22% sand). During the dry period, however, it becomes extremely hard for root expansion, and the water table has no effect on the upper horizons of the soil. The sabkha is inhabited by a multitude of plant species with predominance of two perennial halophytes of the Chenopodiaceae family: *Tecticornia indica* (Willd.) and *Suaeda fruticosa* (Forssk.). Tufts of these halophytes form a microhabitat favorable for the growth of numerous annual glycophytes. These glycophytes constitute a fodder with high nutritional value and significantly contribute to the productivity of the ecosystem, mainly in rainy years (Abdelly *et al*., 1995).

Plant material

The Chenopodiaceae *Tecticornia indica* (Willd.) subsp. *indica* [also called *Artrocnemum indicum* (Willd.) Moq., *Salicornia indica* Willd., and *Halosarcia indica* (Willd.) Paul G. Wilson] is one of the fodder xero-halophytic species appreciated by dromedary, sheep, and occasionally cattle (Le Houérou & Ionesco, 1973). It is a shoot-succulent plant of 20-60 cm in height, common in humid saline regions in Tunisia (Pottier-Alapetite, 1979). *Suaeda fruticosa* (Forssk.) is also a shoot-succulent plant of 30-100 cm in height. It grows in Mediterranean and Atlantic littorals. In Tunisia, it is widespread in semi-arid, arid, and desertic stages and is common in littoral and sabkhas (Pottier-Alapetite, 1979). Weber *et al.* (2007) postulated that *S. fruticosa* seeds contain about 25% oil with 74% unsaturated fatty acids that can be used for human consumption. In addition, shoot aqueous extract of this plant experienced a hypoglycaemic effect in diabetic rats (Benwahhoud *et al*., 2001).

Soil and plant analyses

For soil and plant sampling, we followed the method described by Abdelly *et al.* (1995). In July 2007 (during the driest period of the year) and in February 2008 (when the soil was no longer waterlogged and salt leaching was possible with non-heavy rains), soil samples of the upper 20 cm were taken from four different quadrats of 100 m^2 (10×10 m) chosen in different localities in the sabkha (from the center to the borders). A distance of at least 200 m separated quadrats from each other. In each quadrat, one soil sample was randomly collected from outside halophyte tufts, one from inside of *T. indica* tuft, and one from inside of *S. fruticosa* tuft. Samples were dried, ground, and analyzed for electrical conductivity of the 1/10 aqueous extract ($EC_{1/10}$) and soluble sodium content. For the latter, a Corning flame photometer was used. The aqueous extract was prepared by adding 2.5 g soil to 25 ml H_2O, agitating the suspension for 30 min, centrifuging it for 20 min at 3000 g and then filtering it. In each quadrat, the halophyte tufts were divided into three size categories (small, medium, and big tufts) as described by Abdelly *et al.* (1995). Then two shoot samples were taken from each category (eight replicates for each size category of each species). Shoot samples were dried, ground, and analyzed for sodium and potassium contents using a 0.5% HNO_3 solution for Na^+ and K^+ extraction and a Corning flame photometer for their titration.

Productivity and phytodesalination capacity estimations

The whole shoot biomass of each size category of halophyte tufts was estimated for both species as follows: in each of the four quadrats, one tuft per size class and per species was cut, dried, and weighed. Afterwards, the number of tufts per size class was counted in each quadrat. Hence, halophyte productivities per tuft size were estimated by multiplying the number of tufts by their mean dry weights. Then, taking into account the mean sodium content of each tuft category, we estimated phytodesalination capacity (shoot-accumulated sodium quantity per tuft and per hectare).

Statistical analysis

A one-way ANOVA test of SPSS 10.0 for Windows (SPSS Inc., USA) was used for data statistical analysis and means were compared according to Duncan's test at 5% level of confidence.

RESULTS

Soil analysis

$EC_{1/10}$ values were 2.2-3.5 times higher in inter-tuft than in intra-tuft soil in both July and February samples (Fig. 1). Moreover, in each case, $EC_{1/10}$ values were significantly higher in summer than in winter. For instance, in soil taken from outside halophyte tufts $EC_{1/10}$ was 3.5 dS m^{-1} in July 2007 compared to 2.7 dS m^{-1} in February 2008, which corresponds to ~23% difference. In intra-tuft soil, the decrease between summer and winter was about 55%. The same tendency was found in soluble sodium contents (Fig. 1). For outside tuft the differences in soluble sodium contents between summer and winter samples were not significant (6.2 mg g^{-1} for both). Inside tufts however, soil sodium contents in July 2007 samples were more than twice compared to those measured in February 2008.

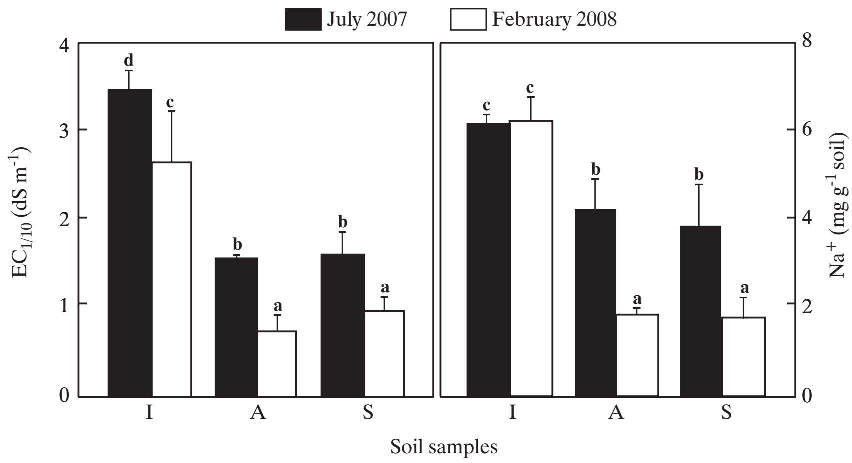

FIG. 1. Electrical conductivities of the 1/10 (w/v) soil aqueous extract ($EC_{1/10}$) and soluble sodium (Na^+) contents in soil samples taken from the upper 20 cm outside (I) or inside *T. indica* (A) and *S. fruticosa* (S) tufts. The sampling was carried out in Soliman sabkha in July 2007 and February 2008. Bars are means of 4 replicates ± standard error. Different letters denote significant differences according to Duncan's test at $a = 0.05$.

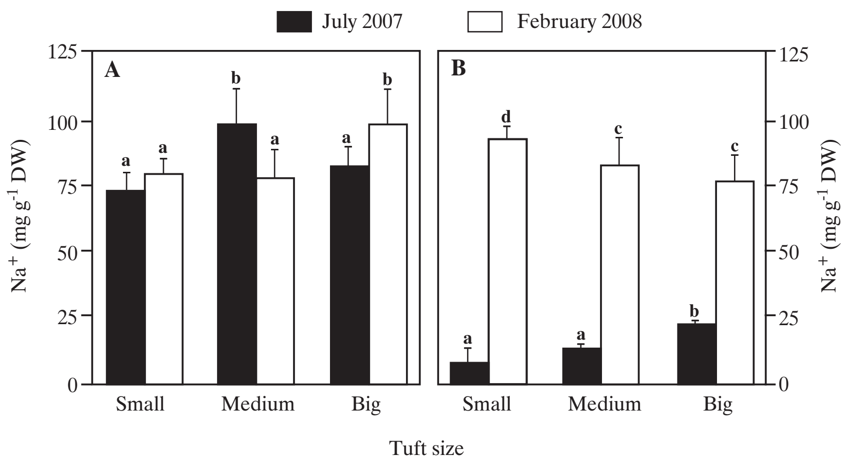

FIG. 2. Shoot sodium contents of small, medium, and big tufts of *T. indica* (A) and *S. fruticosa* (B) collected from Soliman sabkha in July 2007 and February 2008. Bars are means of 8 replicates ± standard error. Different letters denote significant differences according to Duncan's test at $a = 0.05$.

Plant analysis

Sodium content

Shoot sodium content in *T. indica* showed no consistent significant differences according to season (Fig. 2), but in *S. fruticosa*, sodium content in winter was significantly higher than that in summer. As a result, in winter, shoot sodium contents of both species were comparable, varying from 75.7 to 97.3 mg g^{-1} DW (Fig. 2) but in summer, it was 4-12-fold higher in *T. indica* than in *S. fruticosa*. For *T. indica*, the highest value was found in medium tufts collected in summer (about 100 mg g^{-1} DW) and in big ones collected in winter. For *S. fruticosa*, the highest value was found in big tufts sampled in summer and in small ones sampled in winter (25 mg g^{-1} DW).

Potassium content

In *T. indica*, shoot K^+ contents revealed slight variations between July 2007 and February 2008 and the values ranged from 10-13 mg g^{-1} DW with almost no difference between small, medium, and big tufts (Fig. 3A). In *S. fruticosa*, winter shoot K^+ contents were higher than those of *T. indica*, reaching in small tufts 19 mg g^{-1} DW (Fig. 3B), but they exhibited a significant decrease with tuft size. Much lower values were obtained in summer (5-9 mg g^{-1} DW) suggesting detrimental effects of environmental factors on the physiological behavior of *S. fruticosa*.

Productivity estimation

This estimation, performed on the basis of shoot dry weight per tuft, revealed higher capacity of *T. indica* than *S. fruticosa* to produce shoot biomass (Fig. 4A). While the big tufts of the former exceeded 3 kg, those of the latter showed a productivity of 0.6 kg per tuft. The productivity of *T. indica* per hectare was shown to be about 8 times that of *S. fruticosa* (Fig. 4B). This was due to (i) the higher capacity of the former to produce shoot phytomass, and (ii) to its noticeable predominance within the ecosystem. When considering each tuft size apart, we found that for each species, big tufts were responsible for the production of 67-70% of the total biomass. Regardless of species and tuft size, we found a whole productivity of 8.6 tonnes of halophyte shoot per hectare.

Estimation of phytodesalination capacities (shoot-accumulated sodium quantities)

Per tuft

In *T. indica*, shoot-accumulated sodium quantities per tuft were almost the same in summer and in winter for both small and medium tufts (Fig. 5A). For big tufts, higher quantity was observed in February 2008 (337 g Na^+ $tuft^{-1}$) than in July 2007 (261 g Na^+ $tuft^{-1}$). The values estimated in *S. fruticosa* exhibited wide seasonal variations. We found, indeed, that this species was able to accumulate 14, 8, and 4 times more sodium in winter than in summer, in small, medium,

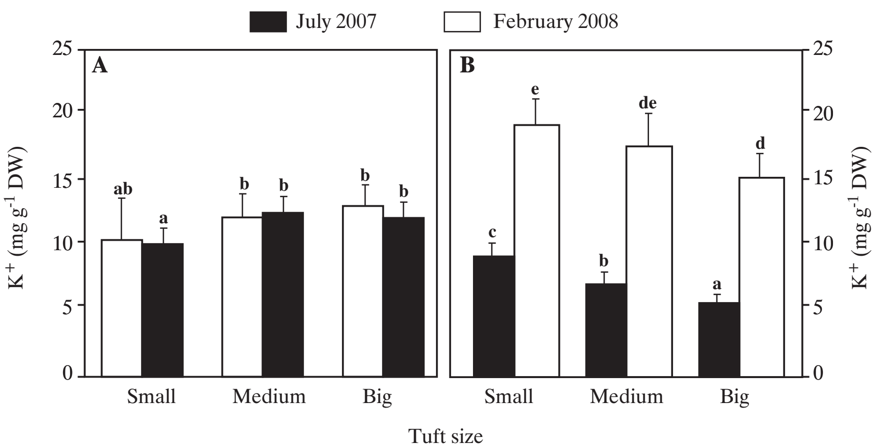

FIG. 3. Shoot potassium contents of small, medium, and big tufts of *T. indica* (A) and *S. fruticosa* (B) collected from Soliman sabkha in July 2007 and February 2008. Bars are means of 8 replicates ± standard error. Different letters denote significant differences according to Duncan's test at $a = 0.05$.

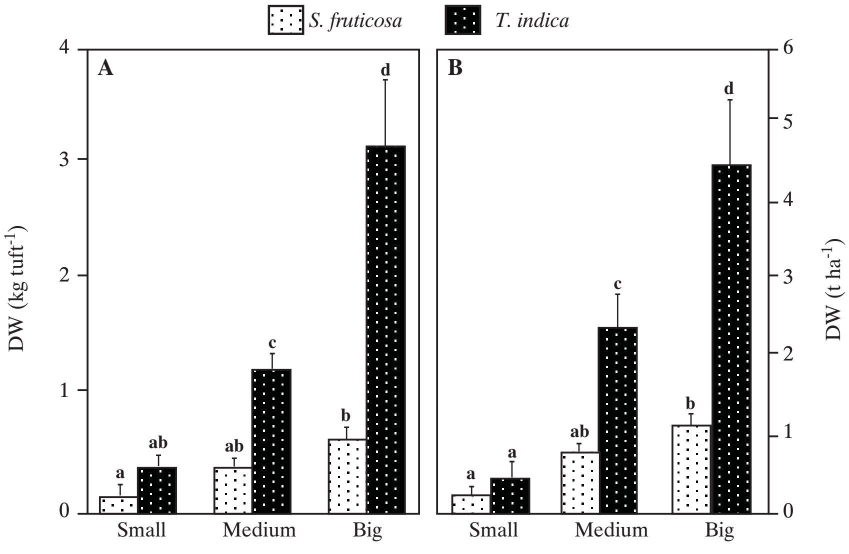

FIG. 4. Productivities by tuft size estimated in terms of shoot biomass per tuft (A) and per hectare (B) in the two chenopodiaceae *T. indica* and *S. fruticosa* collected from Soliman sabkha in February 2008. Bars are means of 4 replicates ± standard error. Different letters denote significant differences according to Duncan's test at $a = 0.05$.

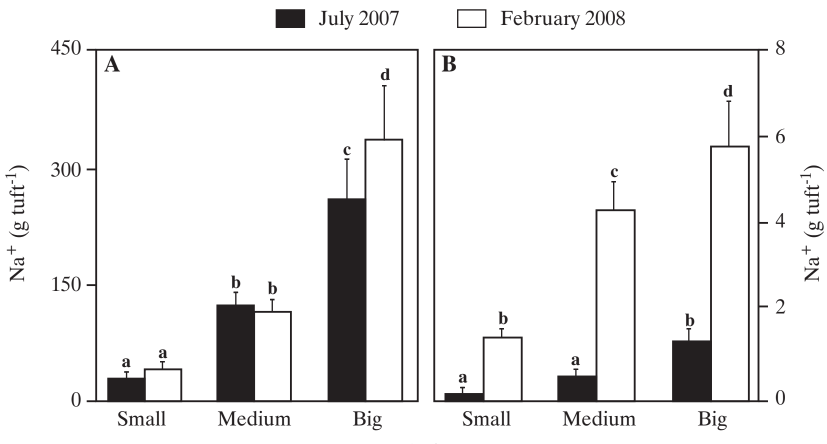

FIG. 5. Accumulated sodium quantities as estimated in terms of g per tuft in shoots of *T. indica* (A) and *S. fruticosa* (B) collected from Soliman sabkha in July 2007 and February 2008. Bars are means of 4 replicates ± standard error. Different letters denote significant differences according to Duncan's test at $a = 0.05$.

and big tufts, respectively (Fig. 5B). But, despite such an increase, these phytodesalination capacities per tuft remained much lower than those of *T. indica*, since they did not exceed 58 g tuft^{-1}.

Per hectare

Our estimations showed that *T. indica* tufts accumulated 0.036, 0.238, and 0.371 tonnes Na^+ ha^{-1}, in small, medium, and big tufts, respectively (Fig. 6A). The increase in the accumulation of these ions in shoots of big tufts resulted in the increase of the total removed quantities per hectare from 0.646 in July 2007 to 0.752 tonnes Na^+ ha^{-1} in February 2008. The estimations showed also that *S. fruticosa* contributed to the process of sodium removing by 22.3% in winter and only 5.6% in summer (Fig. 6B). This suggests a

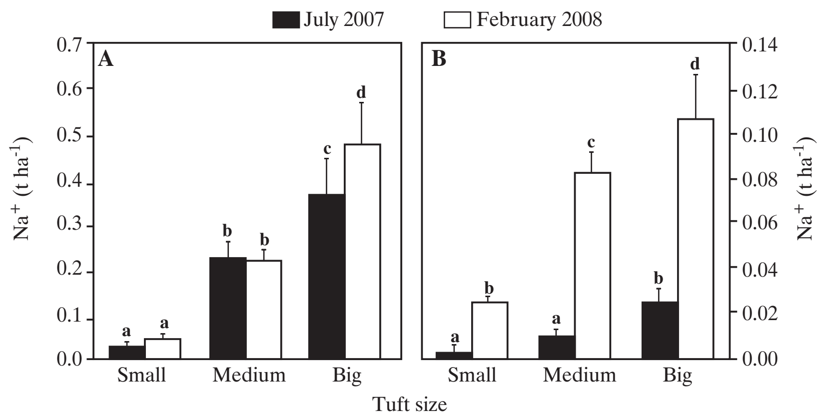

FIG. 6. Accumulated sodium quantities as estimated in terms of tonne per hectare in shoots of *T. indica* (A) and *S. fruticosa* (B) collected from Soliman sabkha in July 2007 and February 2008 (A et B). Bars are means of 4 replicates ± standard error. Different letters denote significant differences according to Duncan's test at $a = 0.05$.

higher phytodesalination capacity of *T. indica* compared to that of *S. fruticosa*. The total quantity of sodium removed by the two halophytes was 0.685 and 0.967 tonnes ha^{-1} in July 2007 and February 2008, respectively.

DISCUSSION

The decrease in the salinity of the intra-tuft soil suggests that the two halophytes were able to desalinize their root zone. This decrease was probably due to a limitation of salt ascension because of their uptake by roots in deep horizons and/or because of a desalination of the upper horizons (Abdelly *et al*., 1995). It could be also the result of salt uptake by superficial roots (Abdelly *et al*., 1995) or the result of the elevation of the soil level inside tufts as a consequence of root expansion, which creates a microrelief within the sabkha (Rabhi *et al.*, 2009). Actually, Abdelly *et al.* (1995) described two different root types in *S. fruticosa* and *T. indica*: 1) deep roots that explore deep horizons limiting in this way the raise of the saline water table and maintaining a less saline upper horizon 2) and superficial roots that absorb sodium from the upper horizon leading to its desalination. The mutual relationship between soil physical and chemical properties and plant growth and development had been shown in many works. For instance, alterations in soil structure due to compaction influence many aspects of the soil such as strength, gas, water and heat, which in turn affect root and shoot growth (Lipiec & Hatano, 2003). Soil salinity (Munns & Tester, 2008) and low nutrient availability (Vysotskaya *et al*., 2008) are also among the major factors affecting plant growth and development. This may explain why annual glycophytes such as *Medicago* species experience higher growth inside than outside tufts of perennial halophytes like *T. indica* and *S. fruticosa* (Abdelly *et al*., 1995). Barazani & Golan-Goldhirsh (2009) found that the evergreen salt tolerant *Pistacia lentiscus* depleted salt in its surroundings which inhibited growth of the halophyte *Salsola inermis*.

We observed also a reduction of the soil soluble sodium content inside tufts, which could partially explain the similar tendency of $EC_{1/10}$ (Fig. 1). Such a correlation between the two parameters was mentioned by Zhao (1991), Ravindran *et al.* (2007) and Rabhi *et al.* (2009) confirmed the high implication of Na^+ ions in soil salinity. Rabhi *et al.* (2009) cultivated *T. indica* and *S. fruticosa* plants over 170 days under non-leaching conditions, on a saline soil taken from the borders of the same sabkha. They observed a decrease of $EC_{1/10}$ from 1.0 dS cm^{-1} in the control (without plantation) to 0.4 and 0.6 dS cm^{-1} in soils desalinized by *T. indica* and *S. fruticosa*, respectively. These decreases were concomitant with similar declines in soluble sodium content which was reduced by more than 50%. The seasonal differences in soil salinity are usually due to the precipitation/water table balance in such saline depressions. In February 2008, the soil samples were collected after the decrease of the level of the saline water table and salt leaching by non-heavy rains in that month. In rainy years, however, the ecosystem remains waterlogged until April-

May, when water evaporation leads to increased salt concentration in the upper horizons of the soil and consequently to increased salinity.

We noticed that in the sabkha, *T. indica* exhibited a higher capacity to accumulate Na^+ ions within its shoots than *S. fruticosa*, mainly in summer. In addition, *S. fruticosa*, which showed during the humid period higher potassium contents than those of *T. indica*, seemed to have a higher aptitude to absorb this nutrient in spite of the excessive sodium concentrations in the soil solution. During this period, *S. fruticosa* plants were more vigorous than in summer when soil drying and high temperature considerably affected their water status and probably their photosynthesis. Thus, some of their leaves were red, senescent, and less succulent. This could explain the reduction in their shoot potassium and sodium contents in the dry season. Abdelly *et al.* (1995) found also that dry shoot parts contained less sodium than growing ones. On the contrary, *T. indica* plants were shown to be more tolerant to the stressing conditions of this season. They maintained higher shoot water contents despite their excessive sodium contents. Under greenhouse conditions, we noticed that root sodium content (2-3 mmol g^{-1} DW) was much lower than that of shoots (about 9 mmol g^{-1} DW) in both species as grown at 100-400 mM NaCl (unpublished data).

Big tufts of *T. indica* and *S. fruticosa*, accumulating biomass over several years, showed shoot dry weights of 3.2 and 0.6 kg $tuft^{-1}$, respectively. Rabhi *et al.* (2009) studied growth of *T. indica* and *S. fruticosa* under greenhouse conditions. Plants were grown on soil taken from their natural biotope and were irrigated with tap water over 170 days. At the harvest, they showed dry weights of 2.11 and 1.85 mg per plant, respectively. Although shoots of *S. fruticosa* tufts showed seasonal variations of sodium content, their contribution to the phytodesalination of the ecosystem was limited in comparison with that of *T. indica* which is more abundant and produces much more phytomass per tuft. These two properties allowed *T. indica* to accumulate more Na^+ ions than *S. fruticosa*. Our estimations showed that the whole shoot phytomass of both species was about 9 tonnes dry weight per hectare containing 0.967 tonnes Na^+ ha^{-1} during the humid period. Such estimations were in agreement with those of Zhao (1991) who noticed that *Suaeda salsa* produced nearly 20 tonnes DW ha^{-1} containing 3-4 tonnes salt. With a less saline soil (4.9 dS m^{-1}), Ravindran *et al.* (2007) found that the capacity of NaCl accumulation of *Suaeda maritima* or *S. portulacastrum* plants over 4 months was 504 and 474 kg per hectare respectively. Observing *S. fruticosa* shoot sodium contents, mainly in summer, one can assume that it is not able to desalinize its rhizosphere. But, soil salinity inside its tufts was similar to that inside *T. indica* tufts. According to Qadir & Oster (2004), the beneficial effect of a plant on its saline rhizosphere, or what they call "vegetative bioremediation", is considered as a function of four main factors: i) partial pressure of CO_2 within the root zone, ii) root proton release in the case of N_2-fixing legumes (these two factors enhance $CaCO_3$ solubility, releasing in this way Ca^{2+} ions that substitute Na^+ ones at the cation exchange site level), iii) root physical effects that improve salt leaching, and iv) shoot Na^+ accumulation. These authors considered that bioremediation of salt-affected soils is mainly based on leaching. Kursakova (2006) found also amelioration in soil physical properties through an enhancement of its porosity under perennial grasses, which favors desalinization of soil profile due to a migration of salts into groundwater *via* root paths. Lucerne (*Medicago* sp.) and sweet clover (*Melilotus suaveolens*) with deep, well-developed root systems experienced the most pronounced effect on the soil desalinization (Kursakova, 2006). The presence of 23% total $CaCO_3$ in the soil of Soliman sabkha supports the hypothesis of sodium leaching. In fact, there are two different opinions about shoot and root effects in soil bioremediation. While Qadir and coworkers (Qadir *et al.*, 1996, 2000, 2002, 2003; Qadir & Oster, 2004) have been attributing such a process to roots, neglecting shoot role, several other authors (Bhatt & Indirakutty, 1973; Zahran & Abdel Wahid, 1982; Zhao, 1991; Helalia *et al.*, 1992; Zhao *et al.*, 2001; Tester & Davenport, 2003; Zhao *et al.*, 2005; Ravindran *et al.*, 2007; Rabhi *et al.* 2009) consider that shoot Na-hyperaccumulating plants can be used efficiently to desalinize saline soils. Taking into account the four factors cited by Qadir & Oster (2004), we can conclude that both *T. indica* and *S. fruticosa* contributed to the bioremediation process of the soil they are growing on, but the former seemed more efficient since it facilitated the leaching of less sodium to the deeper horizons and therefore to the water table. Indeed, to desalinize the upper horizons of the soil by leaching salts is only a short-term solution, as it contributes to the water table salinization. Therefore, the best plants for bioremediation are those showing the highest phytodesalination capacity per hectare.

CONCLUSION

Both *T. indica* and *S. fruticosa* contributed to the phytodesalination of the rhizosphere due to their aptitude to accumulate high sodium quantities within their aerial organs. However, shoot sodium contents of *S. fruticosa* significantly decreased in summer because of a loss of vigor under stressing environmental conditions. This species efficiently decreased soil salinity of the upper horizon by facilitating sodium leaching to the deeper horizons. But, phytodesalination concerns only the fraction of sodium accumulated within the halophyte shoots. When considering each species separately, we noticed that the phytodesalination capacity of *T. indica* was 3.5 and 16.7 times higher than that of *S. fruticosa* in winter and summer, respectively. From the ecological point of view, phytodesalination and the facilitation of sodium leaching in the soil are relevant processes. Indeed, they provide annual glycophytes with a microhabitat favorable for their development, which maintains biodiversity within the ecosystem.

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