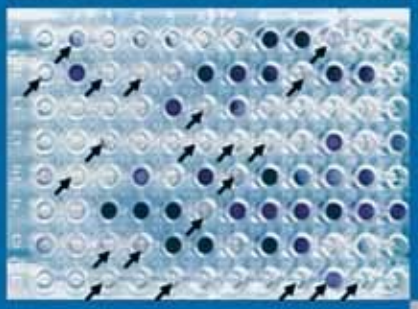

Münir Öztürk
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(Editors)

Biosaline Agriculture and Salinity Tolerance in Plants

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Biosaline Agriculture and Salinity Tolerance in Plants

Edited by M. Öztürk, Yoav Waisel, M. Ajmal Khan and Güven Görk

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Foreword

Global demand for the precious resource of water has increased six-fold over the past century, with a three-fold increase in world population. The water crisis is one of the most critical challenges facing the world today.

Seawater is globally available in abundance, and hypersaline soils are widespread. Based on science, and with carefully established good practices, large areas of saline soils can be converted into high productivity man-made agro-ecosystems.

Substantial information has been provided by numerous scientists since the early 1960s, regarding the restoration, functioning, and development of saline ecosystems and halophytes, and international centres and societies have been established.

We have to be ready to respond when land becomes non-productive due to high salt concentrations. The availability of correct and adequate scientific knowledge is absolutely essential to develop good biosaline management practices.

UNESCO has supported a number of such activities, societies and centres, and this is well in tune with the organisation's focus on *water and associated ecosystems*.

It is now important to identify the next important milestones. A concerted international action is required in order to continue the process of advancing science-based biosaline practices, and to develop profitable models and products. It is also important to raise public awareness: some marketable products have already been developed, and provide valuable services to mankind, such as *Salicornia bigelovii*, *Salicornia europaea*, *Aster tripolium* (vegetable and salad), *Conocarpus erecta*, *Conocarpus lanciofolius* (roadside trees), and *Sesuvium portulacastrum* (to replace freshwater dependant ground cover), to name only a few.

The Arab States in the Gulf suffer greatly from a lack of freshwater availability, whereas saline groundwater and seawater occur in abundance.

It is with this in mind, that the UNESCO Office in Doha, and in agreement with the UNESCO Office in Venice, decided to support Arab experts to participate in *The International Conference on Biosaline Agriculture and Salinity Tolerance in Plants*, Mugla University, Turkey, in January 2005, as well as with this important publication.

The book has three sections: the first section deals with physiological aspects of salt tolerance. It provides data and new information regarding a number of moderate to high salinity tolerant plants species, such as *Vicia faba*, a cash crop, several grass species, as well as *Crithmum maritimum*, *Suaeda salsa*, *Salsola* spp, *Atriplex centralasiatica*, *Cakile maritima*, as well as the seawater tolerant *Sesuvium portulacastrum.*

The second section provides new information on ecological aspects, such as biological diversity conservation, management of natural plant diversity, geographical inventories of halophyte communities, and vegetation zones.

The third section on agriculture provides valuable information on the utilisation of halophytes, soil irrigation and drainage management, bio-reclamation of saline soils, and effects of salinity on crop productivity.

UNESCO congratulates the editors and authors of this book, who produced an excellent scholarly work. Improving the knowledge of the multidisciplinary audience of readers will contribute towards improvement of scientific research, education, and environmental management.

Biosaline Agriculture and Salinity Tolerance in Plants is another important scientific contribution towards the management of salt-affected soils, saline irrigation water, and halophytes.

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The contributions of 127 scientists from 16 countries covering both scientific as well as practical concepts regarding the agricultural production and environmental protection on salt-affected ecosystems is highly appreciated. We gratefully acknowledge the financial support given to us by the Muğla University, Turkish National Research Council, UNESCO-Doha and Toros Gübre-Muğla, Turkey, which made it possible to hold the International Conference on "Biosaline Agriculture and High Salinity Tolerance" in Muğla, Turkey. We would like to express our gratitude to the Governor of Muğla, Mayor of Muğla Dr. Osman Gürün, Rector Muğla University Prof. Dr. Şener Oktik and Vice Rector Prof. Dr. Ibrahim Yokaş, for their keen interest in the organisation of this conference. Finally, our sincerest appreciation goes to the local Organizing Committee in Muğla, in particular Dr. Çiğdem Görk and Dr. Kübra Karaosmanoğlu for their untiring efforts to assure both the scientific and social success of the conference.

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Biosaline Agriculture and Salinity Tolerance in Plants
Edited by M. Öztürk, Y. Waisel, M.A. Khan and G. Görk

Effects of salinity on rhizosphere bacterial communities associated with different root types of *Vicia faba L.*

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Introduction

Soils constitute a heterogeneous and patchy environment that can be divided into innumerable microhabitats. Each of these portray different conditions of temperature, salinity, nutrient composition, abundance and availability, aeration, etc. Conceptually, the various roots of each root system have different environments to adapt to. Such adaptations are manifested by changes in root properties and activities [1].

Comparative investigations of tap and lateral roots of young faba bean plants (*Vicia faba* L.) have shown differences in nutrient uptake, water uptake and growth. Differences were also found in abscisic acid content and in the response of the roots to stimuli by applied hormones. The number of isozymes of several enzymes and their activities differed between taproots and laterals. Such differences were greatly emphasized under stress conditions of salinity, hypoxia and nutrient deprivation [2]. Moreover, it has been shown that the development of such traits is genetically controlled and that a set of genes is specific for each root type [3].

Plant root systems can be classified into different types, each having distinct inherent morphological, physiological and biochemical characteristics [4]. It is postulated that such traits determine the plants' capability to cope with their spatially and temporally heterogeneous environment. It was thus tempting to assume that the physiological differences between roots should also be expressed by changes in their respective rhizospheres at different soil horizons.

Rhizosphere colonization by microorganisms is affected by various environmental conditions [1, 5, 6]. Indeed, root exudates, sloughed-off cells and disintegrating tissues attract bacteria and are the main contributors to the enrichment of the rhizosphere microbiota [6]. Abundance and activity of microbial communities are much higher in the rhizosphere than in the bulk soil [5, 7, 8]. Investigations of rhizosphere bacterial communities were firstly based on culture dependent – but more recently on culture independent – molecular methods [9–12]. Some investigated root-bacteria interactions were found under different environmental conditions, between different

roots and even between different segments along roots [13]. Nevertheless, specificity of association between different root types of individual plants and bacterial communities has hardly been tested.

In the following investigation we tackled the question whether various roots of single root systems of *Vicia faba* support different rhizosphere bacterial communities even when grown under homogeneous saline and non-saline conditions.

Material and methods

Plant growth conditions

Faba bean plants were grown in an aeroponic growth system (25°C, 12 h photoperiod, photon flux: $170\,\mu$mol m^{-2} sec^{-1}) for 18 days. Half strength Hoagland's nutrient solution [14] was supplemented with soil bacterial extracts and 50 mM NaCl was added to some of the treatments. The solution was replaced every 3 days in order to achieve a constant and even supply of nutrients.

Soil samples (20 g samples of a sieved grumosol) were extracted in 80 mL saline solution (0.85% NaCl), shaken and centrifuged. The pellets were re-suspended in saline solution (0.85% NaCl) and aliquots were then added to the aeroponic nutrient solutions. Bacterial cell densities of the soil extracts were determined as colony forming units (CFU) ml^{-1} by plating on Laurie-broth (LB) medium. Cell densities ranged between 4×10^8 and 6×10^9 CFU ml^{-1}.

Root sampling

Roots of 18 days old plants were washed with sterilized water. Terminal 5 cm root segments of the tap and of the lateral roots were transferred into sterile saline solutions (1:10 w:v). Rhizosphere bacterial communities (RBC) were separated from the roots by vigorous vortex for 10 min. The resulting suspensions were diluted ($\times 10^3$) and immediately inoculated into Biolog microplates.

Biolog assay

Biolog GP2 microplates were used for bacterial community analysis. Plates were inoculated with $150\,\mu$L RBC suspensions per well and incubated at 28°C for up to 96 h. Color development was expressed as light absorbance (A) at $\lambda = 595$ nm and was measured at 24 h intervals using the SpectraMAX 190 absorbance microplate reader.

Samples of the obtained RBCs were decimally diluted and plated on LB growth medium and were incubated at 28°C for 4 days. Cell densities were determined and expressed as CFU ml^{-1}. Distinguishable bacterial morphotypes were picked from the plates of each extract and isolated on LB medium.

Taxonomic identification

Taxonomic identification of the conspicuous morphotypes was done using the recommended Biolog system (GP2 and GN2). For a selected number of isolates, 16S rDNA genes were amplified by PCR using the primer set 27f and 1429r [15] (Tab. 1). Sequence results were compared to known 16S rDNA sequences available in the gene bank using the BLAST module.

Data handling and analyses

Biolog GP2 blanked A_{595} of each plate and at each recording time was calculated by subtraction of the blank well reading from each of the carbon source wells. Average well color development (AWCD) values were calculated as: Σ (well A_{595} minus blank well $A_{595}/95$ [16]. Threshold values were set as the minimal A_{595} of the wells in which color could clearly be detected. Binary transformed data was used for non-metric distance analysis (NMDA) using the STATISTICA software package (version 6.0).

Means of the ordination positions were calculated for the 1^{st} and 2^{nd} dimensions in NMDA and compared by two-way ANOVA ($\mathrm{p} < 0.05$).

Diversity of C source utilization in the Biolog plates was analyzed [17]. Shannon-Weiner index of diversity (H′) was calculated as: $\mathrm{H}' = -\Sigma \mathrm{p_i} \ln \mathrm{p_i}$ where p_i is the proportion of color development in the ith well over the total color development of the wells.

Results

Differences between the RBCs of lateral and of taproots were found, both under saline and under non-saline conditions. The root types can be distinguished by differences in the utilization patterns of different carbon source groups as exemplified in Figure 1. These differences were expressed in NMDA (Fig. 2).

The root type strongly affected the RBCs carbon source utilization diversity, expressed by Shannon-Weiner index of diversity (Tab. 2). H′ differed between lateral roots and taproots RBCs whether examined for the complete set of carbon sources or for particular C groups (Tab. 2). Polymers were an exception as H′ of this group was similar between root types and treatments throughout all measurements. The effect of salinity was minor, as compared to the effect of the root type. H′ calculated for the complete set of carbon sources differed between the RBCs of the treatments after 48 h of incubation though not after 72 h and 96 h. The group of sugars was the only carbon group in which H′ of the treatment and control RBCs differed significantly (Tab. 2).

In order to clarify a taxonomic basis for the differences in carbon source utilization patterns, isolated bacterial pure cultures were individually identified using the Biolog GN system. Results indicated higher number of genera for lateral roots RBCs as compared to those of the taproots (Tab. 3). Out of a total of eight identified genera, seven genera were found in the lateral roots RBCs, while only three genera were

Table 1. Oligonucleotide primer sequences and PCR conditions used in this investigation

Target taxon	Primer sequences (forward and reverse)	Target gene	Annealing T°C
Pseudomonas aeruginosa	oprL(f): 5′-ATG GAA ATG CTG AAA TTC GGC-3′ oprL(r): 5′-CTT CTT CAG CTC GAC GCG ACG-3′	*oprL*	64
Herbaspirillum	HRS (f): 5′-GCA AGA CCT CAT GCT CCT G-3′ HRS (r): 5′-CAC GGC TAG AGT GTG TC-3′	*16S rDNA*	62
Eubacteria	27f: 5′-AGA GTT TGA TCC TGG CTC AG-3′ 1492r: 5′- TAC CTT GTT ACG ACT T-3′	*16s rDNA*	56
Eubacteria	519f: 5′-CAG C(A/C)G CCG CGG TAA (A/C/G/T)(A/T)C-3′ 907r: 5′- CCG TCA ATT C(A/C)T TT(A/G) AGT T-3′	*16S rDNA*	53

Table 2. Diversity of carbon source utilization by taproots and by lateral roots rhizosphere bacterial communities of *Vicia faba* in Biolog GP2 microplates. Diversity was expressed as Shannon-Weiner index of diversity (H′) and was calculated after 48, 72 and 96 h of incubation of the microplates for the complete set of carbon sources and for the different carbon source groups. Means of the H′ values of three replicates for each RBC were compared by ANOVA ($p < 0.05$). Means ± standard deviations are presented ($* : P < 0.05$; $** : p < 0.01$; $*** : p < 0.001$).

	Inc. (h)	Root types			Treatments		
		Lateral roots	Taproots		Non-saline	Saline	
Complete set	48	2.74 ± 0.51	3.11 ± 0.17	*	3.11 ± 0.18	2.74 ± 0.51	*
	72	3.21 ± 0.22	3.47 ± 0.14	**	3.39 ± 0.09	3.28 ± 0.31	
	96	3.34 ± 0.18	3.60 ± 0.15	***	3.49 ± 0.07	3.45 ± 0.30	
Sugars	48	1.72 ± 0.72	1.84 ± 0.28		2.07 ± 0.40	1.49 ± 0.50	*
	72	2.25 ± 0.34	2.50 ± 0.17	*	2.46 ± 0.16	2.28 ± 0.37	*
	96	2.41 ± 0.26	2.69 ± 0.23	**	2.58 ± 0.14	2.52 ± 0.38	
Carboxylic acids	48	1.60 ± 0.34	2.01 ± 0.23	*	1.82 ± 0.18	1.79 ± 0.48	
	72	1.94 ± 0.14	2.25 ± 0.15	**	2.07 ± 0.15	2.12 ± 0.28	
	96	2.10 ± 0.10	2.36 ± 0.12	**	2.19 ± 0.14	2.27 ± 0.21	
Amines, amides, amino acids	48	0.92 ± 0.52	1.45 ± 0.12	**	1.33 ± 0.30	1.04 ± 0.56	
	72	1.39 ± 0.17	1.65 ± 0.17	**	1.51 ± 0.09	1.52 ± 0.30	
	96	1.52 ± 0.11	1.77 ± 0.18	**	1.61 ± 0.08	1.67 ± 0.27	
Miscellaneous	48	0.96 ± 0.31	1.51 ± 0.26	*	1.21 ± 0.44	1.26 ± 0.39	
(Unspecified)	72	1.22 ± 0.38	1.90 ± 0.39	*	1.67 ± 0.53	1.45 ± 0.52	
	96	1.30 ± 0.44	1.95 ± 0.39	*	1.66 ± 0.53	1.60 ± 0.56	

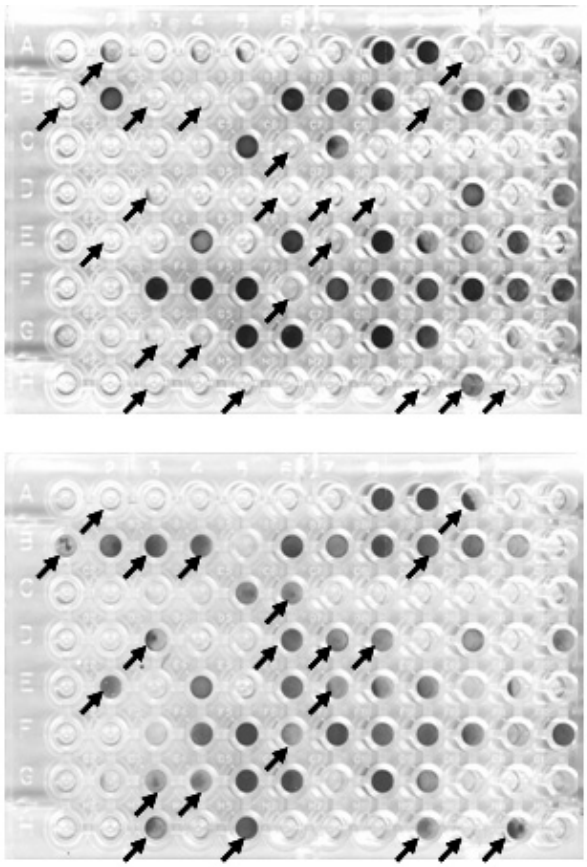

Figure 1. Example of Biolog GP2 Metabolic fingerprints of taproot (top) and of lateral root (bottom) rhizosphere bacterial communities. Arrows indicate qualitative differences in carbon source utilization between the two bacterial communities.

represented by the taproots. Out of these, four genera were represented only in the lateral roots RBCs, while only one genus was unique for the taproots.

Four selected bacterial strains were further identified by sequencing of their 16S rDNA genes. The identified isolates were *Herbaspirillum* sp., *Pseudomonas aeruginosa*, *Burkholderia cepacia* genomovar III and *Pseudomonas alclaigenes*.

Herbaspirillum spp was detected by Real-Time polymerase chain reaction (PCR) only on lateral roots of both control and salt treated plants but not on their taproots (Tab. 4). Relative to the total bacterial targets, the *Herbaspiriilum* spp populations comprised only 0.1% of the bacterial community under non-saline but 0.2% under saline conditions (Tab. 4). *P. aeruginosa* also distinguished between different root types and was found only on taproots (Tab. 4).

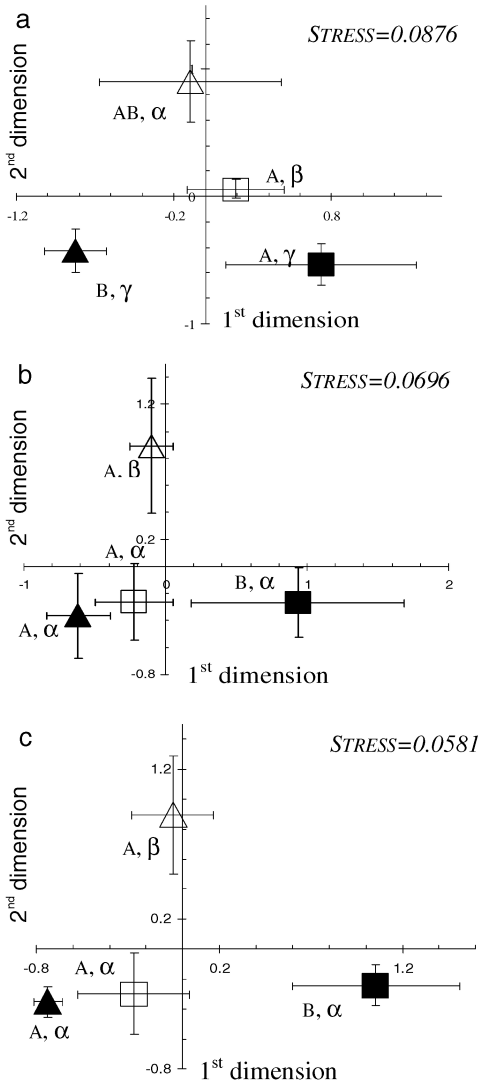

Figure 2. Non-metric multidimensional analysis (A, B and C) ordination of rhizosphere bacterial community (RBC) metabolic fingerprints in Biolog® GP2 microplates (in triplicates) after 48 (A), 72 (B) and 96 (C) hours of incubation. The different RBCs are marked: △ lateral roots of control plants; ▲ lateral roots of salt treated plants; □ taproots of control plants and ■ taproots of salt treatment plants. Stress values of the NMDA ordinations are indicated. Mean coordinates were compared by two-way ANOVA ($p < 0.05$). Different Latin letters indicate significant differences on the 1^{st} dimension. Different Greek letters indicate significant differences on the 2^{nd} dimension.

Table 3. Generic diversity of cultivable bacteria of *Vicia faba* lateral roots and taproots rhizosphere bacterial communities. Conspicuous pure culture isolates were identified using the Biolog® GN2 assay

	Taproots			Lateral roots		
	Total	Gram negative	Gram positive	Total	Gram negative	Gram positive
No. of isolates	35(100%)	30 (85.7%)	5 (14.3%)	71(100%)	54 (76.1%)	17 (23.9%)
Of which identified	12(34.3%)	12	0	21(29.6%)	21	0
No. of genus	3	3	0	7	7	0
Occurrence of genera						
Acidovorax		–			1	
Agrobacterium		–			2	
Burkholderia		2			2	
Comamonas		–			1	
Cytophaga		4			–	
Herbaspirillum		–			6	
Pseudomonas		6			7	
Riemerella		–			2	

Table 4. Real-Time polymerase chain reaction detection of Eubacteria (a), of *Herbaspirillum* spp. (b) and of *Pseudomonas aeruginosa* (c) in rhizosphere bacterial communities of taproots and lateral roots of faba beans (*Vicia faba* L.).

a

Eubacteria				
Sample	Slope: -5.2809; Intercept: 68.932; Coefficient: 0.99			
	Log CFU g^{-1} root f. wt	* Log targets μl^{-1} sample	Log targets g^{-1} root f. wt	Target CFU^{-1}
LC	9.66	6.59	11.94	190
TC	8.94	5.76	11.29	224
LS	8.56	6.07	11.50	871
TS	8.73	6.03	11.81	1202

b *Herbaspirillum*

Sample	Slope: -2.670; Intercept: 41.601; Coefficient: 0.94; Detection limit: 10^1 targets μL^{-1} (cycle 39)				
	Threshold cycle	*Targets μl^{-1} sample	Log targets g^{-1} root f. wt	% of Eubacteria	Melting peak
Positive control	21	6.64×10^7			90.5
LC	34	505	7.93	0.010	89
TC	46	0	0.00	0.000	90.5
LS	35	327	7.82	0.021	89.5
TS	40	0	0.00	0.000	89.5

c *Pseudomonas aeruginosa*

Sample	Slope: -4.238; Intercept: 44.257; Coefficient: 0.987; Detection limit: 10^1 targets μL^{-1} (cycle 39)				
	Threshold cycle	*Targets μl^{-1} sample	Log targets g^{-1} root f. wt	% of Eubacteria	Melting peak
Positive control	21	2.54×10^5			94.5
LC	41	5.9	0.00	0.000	93.5
TC	38	23	7.58	0.020	94.5
LS	40	10.8	0.00	0.000	93
TS	37	51	8.01	0.016	93.5

* Calculated according to the standard curve

Discussion

The root system of faba bean plants (*Vicia faba* L.) is composed of inherently distinct roots that differ biochemically and physiologically [2]. We have hypothesized that such differences have some bearing on the assemblage of distinct bacterial communities associated with different roots.

Reciprocal effects between higher plants and microorganisms have been well demonstrated [18]. Rhizosphere microbial communities differ in abundance and in diversity from bulk soil communities [5, 19–21]. Diversity of rhizosphere microbial communities was shown to be plant-species dependent [5, 22–24]. Moreover, it has been demonstrated that the species composition of soil microbial communities

may determine diversity of the higher plant associations, their productivity and their stability in a given ecosystem [25, 26].

Are such general differences expressed also at a finer level of resolution?

The results presented herein revealed differences between the metabolic fingerprints of rhizosphere bacterial communities of different roots and under different environments. Altogether the results prove that indeed, different roots are associated with distinct rhizosphere bacterial communities.

In order to verify the obtained results and to expand their scope, such distinction was further investigated under salt stress conditions. As with other legumes salinity significantly reduced the growth of *Vicia faba* plants, their nitrogen and protein content [27], and their roots' and leaves' ABA content [4, 28]. Salinity may also affect plant interactions with microorganisms [29]. Thus, to our results, the distinction between the different root types should consider not only the root type but also the environmental salinity.

Previous investigations have shown differences between bacterial communities associated with root segments of different developmental stages of *Cicer arietinum*, *Brassica napus* and *Sorghum bicolor* [5]. It was also found for roots of *Zea mays* [11], and even between cluster roots and non-cluster roots of soil grown *Lupinus albus* [13]. For soil grown plants of *Zea mays*, it was demonstrated that each segment was associated with different assortments of *Burkholderia cepacia* genotypes [30].

Real-Time PCR analysis showed definite root type specific colonization by *Herbaspirillum* spp. *Herbaspirillum* spp are plant growth promoting diazotrophs commonly associated with several gramineous plants [31–37], which were regarded as its 'natural hosts' [34]. Our results support the observations of Valverde et al. [38] and prove that *Herbaspirillum* can also be a common associate of legumes.

A major contribution of the present research is the fact that the results were obtained for roots that were grown under conditions that guarantied uniformity around each and every root of the root system [39]. By that we have proven that distinction between the bacterial communities of the rhizosphere results from inherent differences between the examined root types, and that such differences may be changed by environmental salinity.

Conclusions

The root system of *Vicia faba* is comprised of inherently distinct root types that are associated by characteristic bacterial communities. Such bacterial communities differ not only by the root characteristics but also by the salinity of the environment.

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Biosaline Agriculture and Salinity Tolerance in Plants
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Tolerance of some potential forage grasses from arid regions of Pakistan to salinity and drought

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Introduction

Pakistan falls into arid and semi-arid regions, as about an area of 562,592 km^2 out of 803,935 km^2 of the total area of Pakistan is the arid land with an annual rainfall of less than 60 cm. Of the total salinized land, 56,656 km^2 are saline, 29,138 km^2 are saline sodic and 283 km^2 sodic soil [1]. A large part of available agricultural land remains non-irrigated due to shortage of water. The unavailability of water and low rainfall are the major factors for converting large areas into deserts.

The four main deserts of Pakistan are Thal and Cholistan in the Punjab, Thar in Sindh and Kharan in Baluchistan. Cholistan and Thar are the largest, with annual rainfall of less than 12 cm. The major problems in all deserts are the scarcity of water for irrigation and escalating levels of salts in the soil. The subsoil water in most of the places is brackish.

The twin menace, i.e., salinity and drought, has caused many social and economic problems in the area such as poor living standards, health problems for animals and humans. Selection of salt and drought resistant plant species and their domestication on salt and drought-hit areas is very economic and feasible for overcoming drought and salinity problems [2–4].

Grasses such as *Cenchrus pennisetiformis, Leptochloa fusca, Panicum turgidum* and *Pennisetum divisum* have great importance as forage for livestock and dairy development in deserts. In view of the great economic importance and nutritional value of these grasses, the present study was undertaken to determine the relative resistance to salinity and drought. The major objective of the study was to identify highly salt and drought tolerant species, which may thrive in arid regions under irrigation with subsoil brackish water.

Materials and methods

In the drought experiment, seeds of *Cenchrus pennisetiformis, Panicum turgidum* and *Pennisetum divisum* were collected from the Cholistan and Thal deserts, whereas those of *Leptochloa fusca* were collected from a derelict field. Drought cycles were

started after about 6-week normal growth. The drought treatments maintained during the experiment were control (watering daily to field capacity), mild stress (plants were droughted four times until wilting and re-watered to field capacity), and severe stress (plants were droughted eight times as in mild stress). Various growth, biochemical, and physiological parameters, were recorded during the experiment to evaluate different grasses against drought.

In the salinity experiment, the same grasses as in the drought experiment were used. *Puccinellia distans* was included in this study as it is known for its high salt tolerance. The seed of *Puccinellia distans* was obtained from England. All seed samples were sown in Petri dishes. After 2 weeks the seedlings were transplanted into 18 cm plastic pots containing sand. The salt treatments used were 0, 8, 16 and 24 dS/m, that were prepared by mixing four salts, $NaHCO_3$, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$ and NaCl in a ratio of 1:5:10:30 in half strength Hoagland nutrient solution.

Leaf hydration (H): It is the ratio of the weight of water in a turgid leaf to its dry weight

$H = W_{ref}/W_d$

Where, W_{ref} = Weight in leaf at the reference, W_d = dry weight of the leaf

Leaf elasticity was calculated as:

$E\ (MPa) = (\psi_{w2} - \psi_{w1})/\Delta R$

$\Delta R = (W_1 - W_2)/W_{ref}$

$W_{ref} = W_1 - W_d + (\psi_{w1} - 0.5)\ (W_1 - W_2)/(\psi_{w2} - \psi_{w1})$

Where, W_{ref} = Weight of water in a leaf at the reference, ΔR = change in relative water content, W_1 = Initial weight of leaf, W_2 = Final weight of leaf, W_d = Dry weight of leaf, Ψ_w = water potential of the leaf when over-pressed to –0.5 MPa, Ψ_{w1} = Water potential of the same leaf measured after over-pressing and weighing, Ψ_{w2} = Water potential of the same leaf after over-pressing for 90 seconds.

Osmotic adjustment was calculated by finding the difference between osmotic potential of rehydrated and control plants.

Methods used for the determination of different physiological and biochemical parameters are presented in Table 1.

Table 1. Methods used for the determination of various physiological parameters

Parameter	**Method used**
Proline estimation	Bates et al. [5]
Chlorophyll estimation	Witham et al. [6]
Wax content	Silva Fernandes et al. [7]
Total soluble proteins	Lowry et al., 1951 [8]
Leaf resistance	By porometer (MK3 Delta-T)
Osmotic potential	By vapor pressure osmometer
Analysis of ions	By flame photometer
Leaf elasticity	Thomas [9]

Result and discussion

Drought experiment

Increasing drought cycles had an adverse effect on fresh and dry matter production in all four species (Tab. 2, Fig. 1). Shoot fresh and dry weights in all species decreased consistently at both drought treatments. *Cenchrus pennisetiformis* and *Panicum turgidum* had significantly greater fresh and dry biomass compared with the other species.

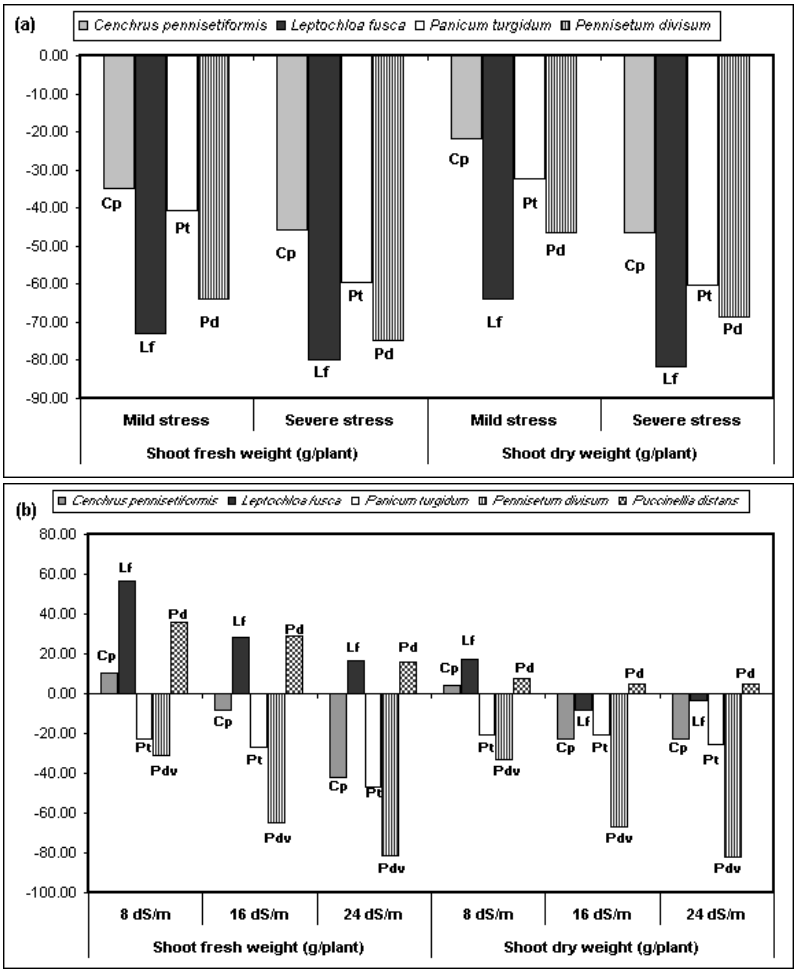

Figure 1. Percent increase or decrease with respect to control of shoot fresh and dry weight of some grasses from Cholistan desert under (a) drought and (b) salinity stress

Thus, *C. pennisetiformis* and *P. turgidum* were relatively resistant to the repeated cycles of drought. In contrast, the lower fresh weight and dry mass production of *L. fusca* shows its susceptibility to drought stress. The relatively greater drought resistance of *C. pennisetiformis* and *P. turgidum* is expected in view of the fact that both species are natural colonizers of the desert area where severe water deficit conditions are predominant [10]. The susceptibility of *L. fusca* to drought is also expected as the species commonly occurs in waterlogged sodic soils [1].

Leaf solute potential decreased significantly in all four grasses with increase in drought cycles. *Leptochloa fusca* had the lowest and *P. divisum* the highest osmotic potential among all the grasses at both drought treatments (Tab. 2). Generally, leaf osmotic potential was more negative in plants which had experienced wilting. This clearly showed that osmotic adjustment was generally increased in plants of all species, which experienced drought cycles. *Leptochloa fusca* had significantly lower osmotic potential but higher osmotic adjustment than the other grasses. Osmotic adjustment plays a central role in plant adaptation to drought [11, 12], although it is known to be effective in plant tolerance to other stress environments such as salinity and freezing [13]. It is now widely accepted that a decline in osmotic potential at full turgor may be due to the apoplastic as well as to accumulation of osmotica [14]. The lower osmotic potentials of plants experienced drought treatments compared with those of plants, which were well watered, may have been due to the accumulation of osmotically active solutes. It thus indicates that osmotic adjustment occurred in all species subjected to drought.

The extent of osmotic adjustment was significantly higher in *L. fusca* and *P. divisum* than the other species (Tab. 2). The results for osmotic adjustment are surprising in view of their contrasting capacity to resist moisture stress. However, there might be some other attributes, which have been contributed to enhance drought resistance in *C. pennisetiformis* and *P. turgidum.* Leaf diffusive resistance in *C. pennisetiformis* which may have been a major factor for controlling water loss, as both *C. pennisetiformis* and *P. turgidum* maintained a non-wilted condition to considerably lower water potential.

Leaf resistance of *C. pennisetiformis* and *P. divisum* increased with increase in drought cycles at morning and evening, whereas that of *L. fusca* and *P. turgidum* remained constant. *Pennisetum divisum* had significantly greater leaf resistance at noon compared with the other three species at 4 drought cycles. Leaf resistance of all the grasses except *C. pennisetiformis* decreased consistently with the increase in drought cycles in evening (Tab. 2).

Cell wall elasticity of *C. pennisetiformis, P. turgidum* and *P. divisum* decreased with the increase in drought cycles, whereas that of *L. fusca* remained unaffected. *Pennisetum divisum* had the lowest and *L. fusca* the highest elasticity among all the species at 4 and 8 drought cycles, respectively (Tab. 2). The elasticity of the cell wall allows volume changes to occur over a range of hydrostatic pressures [15]. The elasticity is an important parameter in cell wall relations, controlling the manner in which cell water potential changes as the cell volume changes [16]. In the present studies, however, drought cycles had no significant effect on elasticity of drought sensitive *Leptochloa fusca,* while it was decreased in other species.

Table 2. Growth, physiological and biochemical parameters of some grasses subjected to mild or sever drought stress

Grass species	Shoot fresh weight (g/plant)			Shoot dry weight (g/plant)			Relative water content (%)		
	Control	Mild stress	Severe stress	Control	Mild stress	Severe stress	Control	Mild stress	Severe stress
Cenchrus pennisetiformis	20.30 d	13.20 a	11.00 a	9.70 b	7.60 ab	5.20 a	71.40 b	70.30 a	87.30 a
Leptochloa fusca	25.30 b	6.80 b	5.10 b	11.40 a	4.10 c	2.10 b	45.80 c	71.00 a	56.60 b
Panicum turgidum	23.70 c	14.10 a	9.60 a	12.10 a	8.20 a	4.80 a	69.40 b	70.50 a	52.40 b
Pennisetum divisum	31.75 a	11.50 a	8.00 a	11.50 a	6.15 b	3.63 ab	90.65 a	86.30 a	81.70 a
	Epicuticular wax content (μg/cm^2)			Leaf osmotic potential (-MPa)			Osmotic adjustment		
Cenchrus pennisetiformis	61.70 bc	158.50 c	153.20 c	1.40 a	2.30 b	2.50 b		0.91	1.11
Leptochloa fusca	34.50 c	281.80 a	321.90 a	1.10 a	2.90 a	3.20 a		1.80	2.12
Panicum turgidum	97.70 b	209.20 b	118.70 d	1.35 a	2.03 b	2.30 bc		0.70	0.95
Pennisetum divisum	185.02 a	183.65 c	222.40 b	0.44 a	1.65 c	2.00 c		1.21	1.50
	Leaf resistance(s/cm)								
	Morning			Noon			Evening		
Cenchrus pennisetiformis	4.10 a	7.30 ab	15.40 a	3.10 b	5.00 b	5.60 a	12.60 a	10.50 a	13.00 a
Leptochloa fusca	4.80 a	6.30 b	7.00 c	3.81 b	3.10 b	3.90 a	10.80 a	9.60 ab	6.00 b
Panicum turgidum	5.50 a	4.64 b	5.70 c	3.55 b	3.70 b	3.54 a	10.90 a	8.00 ab	7.10 b
Pennisetum divisum	3.05 a	11.50 a	11.02 b	11.65 a	13.00 a	5.00 a	13.65 a	7.00 b	8.00 b
	Leaf elasticity (MPa)			Leaf hydration (g water/g)			Proline (μmol/g fresh weight)		
Cenchrus pennisetiformis	6.15 ab	6.20 b	9.90 a	3.60 b	3.00 b	2.90 b	1.65 ab	3.90 b	5.60 b
Leptochloa fusca	5.30 b	5.30 b	6.70 b	5.00 a	4.40 a	2.70 b	3.65 a	6.30 a	8.30 a
Panicum turgidum	5.80 b	7.60 b	10.32 a	3.90 b	3.70 ab	3.40 a	2.04 ab	3.90 b	3.40 c
Pennisetum divisum	8.60 a	12.14 a	12.90 a	3.90 b	3.40 ab	2.40 b	1.01 b	1.00 c	2.95 c
	Chlorophyll a (mg/g fresh weight)			Chlorophyll b (mg/g fresh weight)			Total chlorophyll (mg/g fresh weight)		
Cenchrus pennisetiformis	1.25	1.35	1.35	0.82	0.74	0.74	2.07	2.09	2.09
Leptochloa fusca	1.30	1.30	1.35	1.00	1.00	1.00	2.30	2.30	2.35
Panicum turgidum	1.23	1.20	1.20	0.70	0.80	0.80	1.93	2.00	2.00
Pennisetum divisum	1.10	1.10	1.10	0.60	0.70	0.70	1.70	1.80	1.80
	Total soluble proteins (mg/g fresh weight)								
Cenchrus pennisetiformis	1.20 a	1.10a	1.03 b						
Leptochloa fusca	1.20 a	1.00 a	1.60 a						
Panicum turgidum	1.10 a	1.00 a	1.00 b						
Pennisetum divisum	1.00 a	0.60 b	1.00 b						

Increasing drought cycles significantly decreased the leaf hydration of all the four grasses. *Leptochloa fusca* and *P. turgidum* had the highest leaf hydration at 4 and 8 drought cycles, respectively. The data for relative water content of *C. pennisetiformis* and *L. fusca* increased, whereas it decreased at the highest drought treatment. In contrast, relative water content of *P. divisum* remained unaffected at both drought treatments (Tab. 2).

Increasing drought cycles had no significant effect on chlorophyll a, b, and total chlorophyll content. Species differed significantly for chlorophyll a and b, but this difference was attributed to large differences in their controls. Total soluble proteins of *L. fusca* increased at 8 drought cycles, whereas that of *P. divisum* decreased at 4 drought cycles. In contrast, protein content of *C. pennisetiformis* and *P. turgidum* remained unaffected at both drought treatments (Tab. 2). No significant effect on chlorophyll content in all four grasses may have been to the fact that the specific enzyme responsible for the synthesis of green pigment [17] was not affected by drought. The stability of chlorophyll may also be attributed to the greater accumulation of proline (Tab. 2) and other osmotica. Proline is known to help plants in tolerance of abiotic stresses [18, 19]. Proline forms a hydration sphere around macromolecules and protects them from denaturing under stressful conditions [20–22].

Proline content for *C. pennisetiformis* and *L. fusca* increased significantly, whereas that of *P. turgidum* and *P. divisum* remained unaffected at both drought treatments. *Leptochloa fusca* synthesized significantly greater amount of proline at both treatments compared with the other species (Tab. 2). Leaf proline content generally increased with increase in drought intensity, but greater increase was recorded in drought susceptible *L. fusca*. The results clearly show that proline levels in the drought sensitive species were inversely correlated with the ability to withstand severe drought.

Leaf epicuticular wax of *L. fusca* and *P. turgidum* increased consistently with increase in drought intensity, whereas that of *C. pennisetiformis* increased only at the mild stress level. Leaf wax content of *P. divisum* remained unaffected at both drought treatments (Tab. 2). Deposition of wax on leaf surface plays a crucial role in minimizing water loss [23]. Although all the plants accumulated considerable quantity of wax under drought conditions, *L. fusca* and *P. divisum* showed the highest epicuticular wax content, which indicates a negative correlation to drought resistance. However, Ashraf and Mehmood [24] reported a positive correlation between deposition of wax on leaf surface and drought resistance in some *Brassica* species.

On the basis of these results it can be concluded that *C. pennisetiformis* and *P. turgidum* were the most drought tolerant, *P. divisum* being intermediate and *L. fusca* the most sensitive to drought stress. However, very few relationships were observed between the drought tolerance of these grass species and different physiological attributes examined in this study.

Salinity experiment

The five grass species *Leptochloa fusca, Cenchrus pennisetiformis, Panicum turgidum, Pennisetum divisum* and *Puccinellia distans* responded differently to increasing

salinity level of rooting medium. Increasing salinity treatment markedly reduced shoot and root dry weights in *C. pennisetiformis, P. turgidum* and *P. divisum*, whereas those of *L. fusca* and *P. distans* showed stability at all salinity treatments (Tab. 3). *Leptochloa fusca* had significantly greater shoot fresh and dry matter than the other grass species at 16 and 24 dS/m. Percent fresh and dry matters of *L. fusca* and *P. distans* remained unaffected at all salt treatments (Tab. 3). In contrast, percent biomass production in *P. divisum* was severely reduced by salinity (Fig. 1). The results for the biomass production clearly shows that *L. fusca* and *P. distans* were highly tolerant to varying salinity levels of the growth medium compared with the other three species. The better performance of these two species is expected as they were already found to be highly salt tolerant, e.g., *L. fusca*[1], and *P. distans* [2]. *Pennisetum divisum* was the poorest of all species, whereas *C. pennisetiformis* and *P. turgidum* were intermediate in performance in response to salinity.

Leaf osmotic potential of all five species except *L. fusca* decreased with increase in salt concentration. *Puccinellia distans* and *P. turgidum* had the lowest osmotic potential at 8 and 16 dS/m, respectively, whereas *L. fusca* had the highest osmotic potential at 24 dS/m of all the five species. The remaining species did not differed significantly at all salt treatments (Tab. 3).

Salinity treatments had no affect on leaf soluble proteins of all the grass species while proline content increased with increasing salinity treatments. Proline content of *L. fusca* and *P. distans* increased consistently with increasing stress level whereas, that of *C. Pennisetiformis*, *P. turgidum*, and *P. divisum* remained unaffected at all the treatments. *Puccinellia distans* had significantly greater amount of proline compared with all the other species at all salt treatments. *Leptochloa fusca* also synthesized greater quantity of proline than the remaining three species at the higher salt treatments. The proline content of the remaining species was almost similar at all salt treatments (Tab. 3).

The high osmotic potential of *L. fusca* are less easy to explain in view of its considerable salt tolerance and high accumulation of proline in the leaves, as proline has been reported to be osmotically very active [25]. However, there was a positive correlation between low osmotic potential and high proline content in *P. distans*. In contrast, the low osmotic potential values in the other three species cannot be explained in view of the organic solutes analyzed in this study. It is possible that sugars, organic acids or other quaternary ammonium compounds that have not been determined in this study might have played a role in maintaining low osmotic potential.

Increasing salt concentration in the rooting medium had no effect on relative water content of all the five grass species. The maximum water content of all grasses was observed at 16 dS/m while it was considerably lower in other treatments. *Cenchrus pennisetiformis* showed the maximum water content at this treatment followed by *P. divisum* and *P. turgidum* (Tab. 3).

The mean data for chlorophyll (Tab. 3) showed that increasing salt intensity had no effect on chlorophyll a, b, and total chlorophyll. *Leptochloa fusca* and *P. divisum* had higher Chl. b than the other species at 8 dS/m. In contrast, at 24 dS/m *L. fusca* and *P. turgidum* had greater Chl. b compared with the other species. *Leptochloa fusca*

Table 3. Effect of salinity on some growth, physiological and biochemical parameters of some grasses

Grass species	Shoot fresh weight (g/plant)				Shoot dry weight (g/plant)				Root dry weight (g/plant)			
		dS/m				dS/m				dS/m		
	Control	8	16	24	Control	8	16	24	Control	8	16	24
Cenchrus pennisetiformis	21.6 bc	23.9 ab	19.8 ab	12.5 b	9.2 b	9.6 a	7.1 ab	7.1 ab	5.3 ab	4.7 bc	5.2 b	3.0 b
Leptochloa fusca	18.2 c	28.5 a	23.4 a	21.2 a	8.8 b	10.3 a	8.1 a	8.5 a	3.6 bc	3.1cd	2.2 c	1.7 c
Panicum turgidum	27.7 b	21.4 bc	20.3 ab	14.8 b	9.3 b	7.4 a	7.4 ab	6.9 ab	7.2 a	8.1 a	10.5 a	7.1 a
Pennisetum divisum	44.3 a	30.5 a	15.7 b	8.2 b	16.5 a	11.0 a	5.5 b	3.0 c	6.0 a	5.5 b	2.2 c	2.3 b
Puccinellia distans	11.5 d	15.6 c	14.8 b	13.3 b	4.0 c	4.3 b	4.2 c	4.2 b	1.9 c	2.7 d	1.3 c	1.8 bc
	Leaf resistance (s/cm)											
	Morning				Noon				Evening			
Cenchrus pennisetiformis	5.2 a	11.3 a	7.0 a	5.9 b	2.2 a	2.9 a	6.2 b	4.0 b	9.7 a	9.4 a	10.1 b	15.4 b
Leptochloa fusca	5.6 a	3.9 a	4.8 a	4.8 b	4.6 a	3.8 a	3.2 b	4.2 b	5.7 a	6.7 a	11.8 b	14.6 b
Panicum turgidum	6.1 a	6.9 a	5.6 a	1.2 b	2.9 a	2.5 a	4.3 b	4.1 b	7.3 a	6.3 a	9.6 b	11.5 b
Pennisetum divisum	3.9 a	7.4 a	10.4 a	15.5 a	4.2 a	2.3 a	16.2 a	13.1 a	9.8 a	12.3 a	30.1 a	36.0a
Puccinellia distans	3.3 a	2.6 a	3.8 a	2.7 b	2.0 a	1.8 a	2.8 b	1.7 b	3.7 a	4.3 a	8.9 b	3.4 b
	Leaf osmotic potential(-MPa)				Relative water content (%)				Epicuticular wax content (μg/cm^2)			
Cenchrus pennisetiformis	0.8 a	2.4 b	2.4 b	5.0 a	72.1	61.4	80.4	67.2	37.0 d	113.9 c	102.3 b	219.1 b
Leptochloa fusca	0.9 a	1.8 b	2.0 b	2.0 c	52.1	53.9	77.6	49.3	248.2 b	252.7 b	207.0 a	301.0 a
Panicum turgidum	0.9 a	1.7 b	5.5 a	5.3 a	65.4	61.3	75.1	62.1	73.5 d	126.7 c	263.1 a	87.1 c
Pennisetum divisum	0.4 a	0.9 b	2.0 b	4.0 b	66.2	66.3	78.5	50.7	139.3 c	318.5 a	84.4 b	80.0 c
Puccinellia distans	1.6 a	3.3 a	2.3 b	3.6 b	64.8	57.2	78.3	58.5	321.6 a	167.8 bc	233.6 a	289.1 a
	Chlorophyll a (mg/g f. wt.)				Chlorophyll b (mg/g f. wt.)				Total chlorophyll (mg/g f. wt.)			
Cenchrus pennisetiformis	0.90	1.3	1.30	1.20	0.45 b	0.70 c	0.90 a	0.50 c	1.40 c	2.30 c	2.30 b	1.80 c
Leptochloa fusca	1.40	1.40	1.35	1.30	1.20 a	1.30 a	1.13 a	1.40 a	3.00 a	3.30 a	3.00 a	2.70 a
Panicum turgidum	1.30	1.30	1.33	1.30	0.90 a	1.00 b	0.90 a	1.11 b	2.50 b	2.60 bc	2.60 ab	2.80 a
Pennisetum divisum	1.10	1.05	1.20	1.20	0.60 b	0.50 c	0.60 b	0.60 c	1.75 c	1.70 d	2.10 b	2.10 bc
Puccinellia distans	1.30	1.30	1.30	1.30	1.30 a	1.10 ab	1.10 a	1.10 b	3.00 a	2.90 ab	2.60 a	2.34 ab
	Total soluble proteins (mg/g f. wt.)				Proline (μmol/g f. wt)							
Cenchrus pennisetiformis	0.9	1.2	0.9	1.0	3.3 b	3.2 b	1.6 b	2.9 c				
Leptochloa fusca	1.0	1.1	1.0	1.1	2.6 b	2.6 b	10.0 b	33.4 b				
Panicum turgidum	0.9	1.2	1.1	1.3	3.0b	3.0 b	2.7 b	3.2 c				
Pennisetum divisum	1.1	1.3	0.9	0.9	1.4 b	2.4 b	1.6 b	4.7 c				
Puccinellia distans	1.4	1.1	1.2	1.3	38.5 a	47.2 a	83.6 a	83.6 a				

also had greater total chlorophyll at all salt treatments, whereas *P. divisum* and *C. pennisetiformis* had lower total chlorophyll than the other species at 8 and 24 dS/m, respectively. No significant effect on chlorophyll content in all five grasses may have been due to specific enzyme responsible for the synthesis of green pigment [17]. The stability of chlorophyll may also be attributed to the greater accumulation of proline (Tab. 3) and other osmotica. Proline is known to help plants in tolerance of abiotic stresses [18, 19]. Proline forms a hydration sphere around macromolecules and protects them from denaturing under stressful conditions [20–22].

The leaf diffusive resistance of *P. divisum* determined in the morning increased consistently with the increase in the salt treatment, whereas those of all other four species remained almost consistent. *Pennisetum divisum* had highest leaf resistance of all five species in the morning at 24 dS/m. Leaf resistance of *P. divisum* measured at noon increased with increasing salt treatments, whereas those of other four species remained almost unchanged at all salt treatments except *C. pennisetiformis* that had significantly greater leaf resistance at 16 dS/m than its control. *Pennisetum divisum* had the highest, whereas *P. distans* the lowest leaf resistance at 16 and 24 dS/m. The leaf resistance of *L. fusca* and *P. divisum* increased, whereas those of *C. Pennisetiformis, P. turgidum and P. distans* remained unaffected at all salt treatments in the evening (Tab. 3).

A consistent increase in deposition of wax on leaf surface was observed in *L. fusca* and *P. distans* with increase in salt concentration of the growth medium. *Pennisetum divisum* was the lowest and the other two species intermediate in the deposition of wax. The results for epicuticular wax content of the five species can easily be correlated with their tolerance to salt stress. It is now well evident that epicuticular wax content on leaf epidermis plays a pivotal role in minimizing evaporative loss and thus maintaining high turgor [23]. However, the results for epicuticular wax cannot be correlated with the leaf resistance data of the five species, because *P. divisum* with low wax deposition had considerably high leaf resistance at the higher salinity treatments. In contrast, *P. distans* with excessive epicuticular wax was the lowest in leaf resistance among all species (Tab. 3).

Increasing external salt concentration affected shoot Na^+ concentration non-significantly (Tab. 4). *Cenchrus pennisetiformis* and *P. divisum* had significantly higher and *L. fusca* and *P. distans* lower shoot Na^+ at 16 dS/m, but at higher salinity (24 dS/m the difference in Na^+ concentrations in all the species was non-significant. Root Na^+ of all five species increased with increase in salt level. *Panicum turgidum* had significantly higher concentration than the other species at all salt treatments. At 8 dS/m *P. divisum* and *L. fusca* had intermediate and *C. pennisetiformis* and *P. distans* the lowest root Na^+.

Cl^- concentrations in the shoots and roots in all five species increased with increase in salt treatment except in shoot of *C. pennisetiformis*, which had almost equal to its control at 8 and 24 dS/m, respectively. *Puccinellia distans* and *P. divisum* had significantly higher shoot Cl^- than the other species at 8 and 16, and 24 dS/m, respectively. *Panicum turgidum* had the highest root Cl^- concentration of all five species at all salt treatments (Tab. 4).

Table 4. Effect of salinity on ion accumulation of some grasses

Grass species	Na^+mmol kg^{-1} fresh weight)				Cl^- (mmol kg^{-1} fresh weight)			
		dS/m				dS/m		
	Control	8	16	24	Control	8	16	24
	Shoot				Shoot			
Cenchrus pennisetiformis	194.3 ab	236.4 a	245.5 a	228.6 ab	193.1 a	186.2 b	287.7 ab	227.0 c
Leptochloa fusca	213.5 a	260.5 a	112.2 b	287.2 a	176.7 a	258.0 b	238.3 b	484.0 a
Panicum turgidum	248.1 a	213.9 a	88.2 b	194.3 b	129.8 a	222.9 b	249.0 b	290.4 bc
Pennisetum divisum	145.5 c	225.2 a	277.3 a	257.4 a	148.0 a	224.6 b	240.1 b	598.2 a
Puccinellia distans	178.6 b	105.8 b	145.9 b	180.2 b	136.4 a	370.4 a	372.5 a	349.0 b
	Root				Root			
Cenchrus pennisetiformis	31.8a	31.8 c	158.6b	249.8 b	30.4 a	109.6 b	164.9 b	126.0 b
Leptochloa fusca	34.1 a	141.6 b	129.8 b	169.6 b	42.9 a	75.6 c	82.1 c	152.6 b
Panicum turgidum	48.9 a	238.3 a	254.6 a	503.5 a	88.0 a	162.1 a	217.1 a	440.4 a
Pennisetum divisum	27.4 a	22.9 c	156.7 b	189.6 b	42.2 a	83.4 bc	85.4 c	185.0 b
Puccinellia distans	39.5 a	32.1c	109.3 b	167.5 b	56.3 a	55.6 c	90.2 c	179.3 b
	Ca^{2+} (mmol kg^{-1}fresh weight)				K^+ (mmol kg^{-1}fresh weight)			
	Shoot				Shoot			
Cenchrus pennisetiformis	148.4 a	115.6 a	194.7 a	153.2 b	212.1 a	136.4 ab	100.5 a	108.2 b
Leptochloa fusca	144.8 a	105.2 a	140.0 b	219.8 a	205.5 a	133.1 ab	98.6 a	169.0 a
Panicum turgidum	82.4 b	106.6 a	114.6 c	164.9 b	262.4 a	152.3 a	129.1 a	137.9 ab
Pennisetum divisum	101.6 b	58.7 b	146.0 b	118.6 c	216.7 a	117.9 ab	149.2 a	124.1 ab
Puccinellia distans	90.8 b	90.3 a	148.2 b	116.7 c	137.9 b	103.4 b	131.3 a	94.0 b
	Root				Root			
Cenchrus pennisetiformis	66.5 d	56.0 c	114.8 a	120.2 ab	32.6 b	31.7 b	36.8 c	29.1 c
Leptochloa fusca	89.7 c	85.2 b	47.3 b	133.3 a	43.4 a	39.0 ab	37.0 c	60.4 b
Panicum turgidum	132.2 a	209.9 a	123.0 a	107.4 b	85.8 ab	41.6 a	102.9 a	136.4 a
Pennisetum divisum	104.8 bc	56.7 c	51.8 b	84.2 c	31.9 ab	30.0 b	65.0 b	13.3 c
Puccinellia distans	114.2 b	76.7 b	65.8 b	109.9 b	51.6 b	59.4 a	33.9 c	60.7 b

Shoot K^+ concentrations (Tab. 4) of all species decreased significantly with increase in salinity level, whereas root K^+ of all species remained unaffected. *Pennisetum divisum* and *L. fusca* contained relatively higher shoot K^+ at 16 and 24 dS/m, respectively. *Puccinellia distans* showed relatively lower shoot K^+ at 8 and 24 dS/m. *Panicum turgidum* had the highest root K^+ of all five species at 16 and 24 dS/m.

Shoot Ca^{2+} concentrations of *P. turgidum* and *P. distans* increased with increase in salinity level, whereas the remaining three species did not show any consistent pattern of increase or decrease in shoot Ca^{2+}. *Cenchrus pennisetiformis* and *L. fusca* accumulated relatively greater amount of Ca^{2+} in the shoots than the other species at 8 and 16, and 24 dS/m, respectively. Increasing salinity had no significant effect on root Ca^{2+} of all five species. *Panicum turgidum* had significantly greater concentrations of root Ca^{2+} compared with the other species at 8 and 16 dS/m (Tab. 4).

In the present study the salinity treatments were prepared by mixing different salts in ratios that correspond to the composition of subsoil saline water from the deserts. Therefore, considerable interaction of different ions in all species has been observed at each salinity level. Each species used its own specific selective ion

transport mechanism in response to varying salinity treatments. For instance, the highly tolerant *Leptochloa fusca* accumulated relatively greater concentrations of Na^+ and Cl^- in the shoots at the highest salinity level, whereas these concentrations were low in the roots. Thus, the species used a typical halophytic mechanism [26]. Since the species possesses characteristic salt glands [1] it is possible that Na^+ and Cl^- absorbed by roots are rapidly translocated to leaves for onward excretion through salt glands. *Leptochloa fusca* also accumulated high concentrations of both K^+ and Ca^{2+} for maintaining Na^+/K^+ and Na^+/Ca^{2+} ratios low in the shoots. High Na^+/K^+ and Na^+/Ca^{2+} ratios have already been found responsible for increasing membrane permeability in plants [27, 28].

In contrast, the second highly salt tolerant species, *P. distans* partially included Na^+ from both shoots and roots, but accumulated high concentration of Cl^- in the shoots. The low concentrations of both Na^+ and K^+ in the shoots of *P. distans* show that it does not show selectivity to both K^+ and Na^+, as was suggested by Greenway and Munns [27] that some mesophytes are selective to K^+ while others are selective to both K^+ and Na^+.

The relatively most salt sensitive species, *P. divisum* showed a clear relationship between its poor growth and patterns of ion accumulation under saline conditions. Its high accumulation of both Na^+ and Cl^- in the shoots can be related to the early findings of Wyn Jones et al. (1984) [25] who demonstrated that *Agropyron intermedium* was salt sensitive compared with *Agropyron junceum* because it efficiently accumulated both Na^+ and Cl^- in its leaves. In addition, low Na^+/K^+ and Na^+/Ca^{2+} ratios were not maintained by this species at varying salinity treatments.

The other two species, *C. pennisetiformis* and *P. turgidum* were relatively intermediate in salinity tolerance. *Cenchrus pennisetiformis* absorbed large amount of Na^+ in the shoots, but at the same time it accumulated high concentrations of Ca^{2+} in shoot so as to maintain Na^+/Ca^{2+} ratio low. In contrast, *P. turgidum* maintained low concentrations of both Na^+ and Cl^- in the shoots, although the concentrations of these ions were high in its roots. This type of mechanism is very common in many salt tolerant mesophytes ([27, 29, 30] Netondo et al., 2004). The same authors advocated that salt excluders have the ability to restrict the uptake of salts into the shoot. This might be due to the phenomenon that toxic ions such as Na^+ are absorbed in considerable amount, but are reabsorbed from the root or the shoot and is either stored in the roots or retranslocated to the soil.

Taken overall, it is not difficult to say that *C. pennisetiformis* and *P. turgidum* intermediate in salt tolerance can be grown in those drought hit areas having moderately saline subsoil water for irrigation. These species have high forage value for all types of livestock and already well adapted to the prevailing environmental conditions of the area[10]. Thus, these two species could be of great value for economic utilization of the desert area. The other two highly salt tolerant species *L. fusca* and *P. distans* may not be suitable for the area as they both are adapted to entirely different environmental conditions than those of deserts and also are highly sensitive to drought conditions.

With the proper management of arid lands, the production of these grasses can be enhanced which can bring socio-economic gain of the farmers rehabilitated in

these areas. Our present study also confirms that these grasses can be introduced on salt affected and drought prone areas where livestock is facing severe problems of fodder shortage.

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Salt and seawater effects on the germination of *Crithmum maritimum*

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Introduction

Crithmum maritimum (*Apiaceae*), also called sea fennel, is a perennial halophyte that thrives on saline environments (rocky coasts, piers and breakwaters) along the Mediterranean countries, Pacific and Atlantic coasts [1]. Several uses are known for this plant: for culinary purposes, fresh leaves and young branches are pickled in vinegar and used as condiments. Leaves have also medicinal applications, as antiscorbutic, tonic, diuretic, and vermifuge substances [2].

Salt tolerance during the germination step is critical for the successful establishment of the future plant. Vascular species often show a delay and a reduction in germination as salt levels in the medium increase [3]. The reduction in germination may be due to the low osmotic potentials occurring under saline conditions or to the toxic effects of the ions. Nevertheless, an important characteristic that distinguishes halophytes from glycophytes is their ability to remain dormant at high salinities and to germinate later when the level of salinity is reduced.

We studied in the present work the germination response of *C. maritimum* seeds to NaCl and sea water, as major salinity agents, in order to identify the threshold salinity for a significant reduction in germination.

Material and methods

Seeds of *C. maritimum* were collected from Tabarka (North West of Tunisia) and kept dry in a cold room at 4° C. Sterilised seeds were germinated in Petri dishes, on filter paper moistened with either distilled water or saline solutions containing either NaCl (50, 100, 150, 200, 300 mM) or sea water at different dilutions (5, 10, 20, 30 %). Four replicates of 25 seeds each were used for each treatment. Seeds were considered to germinate at the emergence of the radicle. Seed germination was recorded each 2 days for 46 days. Seeds which failed to germinate in saline solutions were transferred in distilled water to assess seed viability.

Germination rate was calculated using a modified Timson index of germination velocity; $\sum G/t$, where G is the percentage of seed germination after 2 d intervals,

and t is the total time of germination [4]. Rate of recovery of germination was calculated using the relation $[(a-b)/(c-b)]$ 100, where a is the total number of seeds germinated after beg transferred to distilled water, b is the total number of seeds germinated in saline solution, and c is the total number of seeds.

A one-way variance analysis (ANOVA) was carried out by the statistical software SPSS 10.0.

Results

The evolution of the germination percentage of *C. maritimum* over the salt-treatment period showed that the germination process was strongly inhibited when sea water dilution exceeded 5 % (Fig. 1). Sea water at 5 % delayed the germination but enhanced the germination plateau up to 85 % after 20 days, while reaching 75 % in distilled water. The variation of the germination capacity of *C. maritimum* in the presence of NaCl showed that the germination was delayed under increasing NaCl, and strongly inhibited at NaCl concentrations higher than 50 mM. Oneway ANOVA revealed that the final germination percentage was significantly inhibited by both sea water and NaCl (Figs. 2A and 2B). The same pattern appeared when considering the germination rate (Figs. 2C and 2D), thus confirming the salt-induced germination delay.

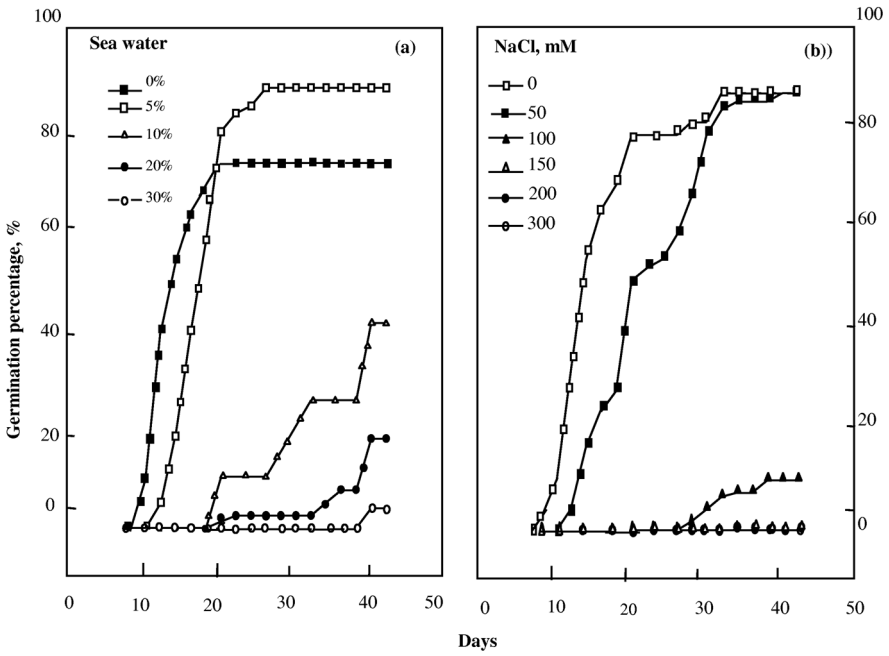

Figure 1. Effect of salinity (**a**: sea water, **b**: NaCl) on the germination (%) of *Crithmum maritimum*

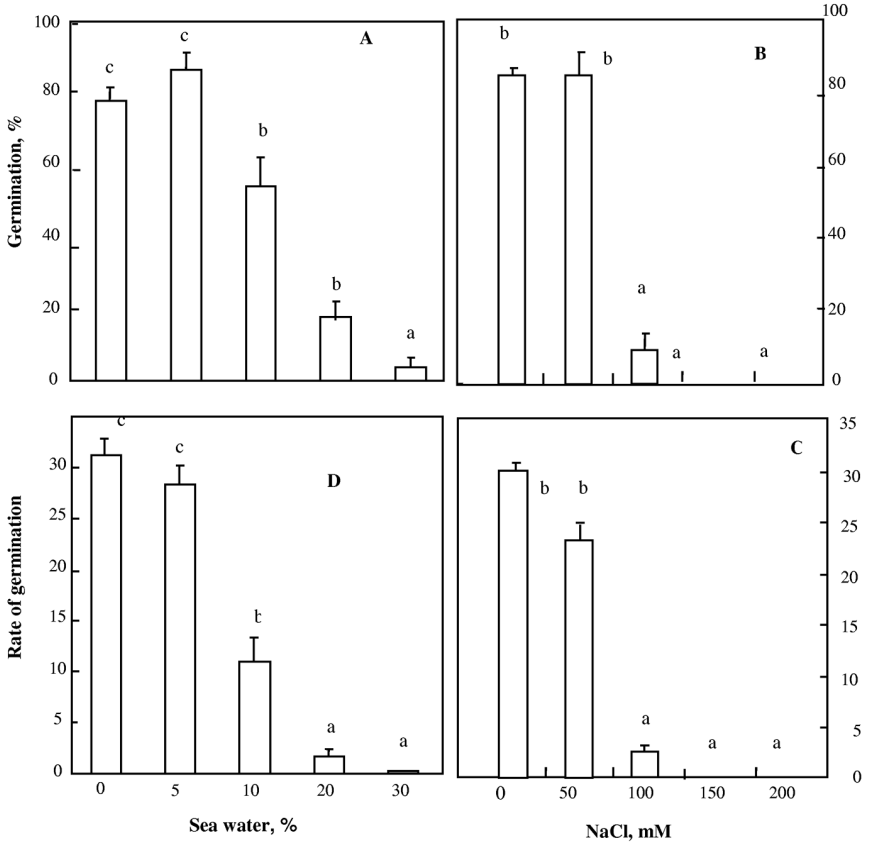

Figure 2. Effect of salinity on germination parameters of *C. maritimum*. **A** and **B**: mean final germination percentage under sea water and NaCl, respectively. **C** and **D**: Rate of germination (Timson's germination velocity index) under sea water and NaCl, respectively. The two parameters were determined after 46 days of treatment. Different letters indicate statistically significant differences ($P < 0.05$) between treatments (by Tukey's test).

The inhibition of germination could be due either to salt-induced seed mortality, or to unfavourable external osmotic conditions. In order to distinguish between these two factors, the seeds which did not germinate in the presence of salt were transferred on pure water. Nine days later, up to 90 % germination recovery was observed (Fig. 3), which suggests that NaCl did not alter seed viability. Thus, the observed inhibition of germination following NaCl treatment seems to be mainly attributable to unfavourable osmotic conditions, although NaCl toxic effect was observable at the highest concentrations.

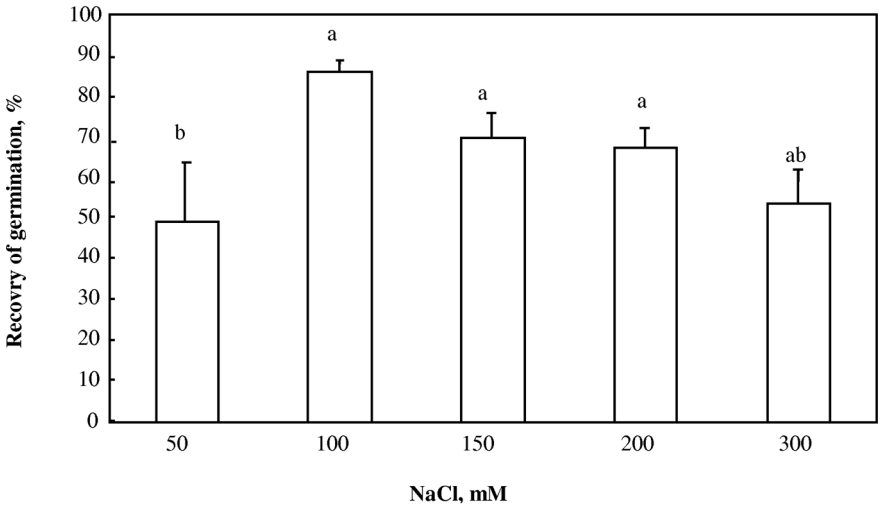

Figure 3. Germination recovery (%) of *C. maritimum*. All seeds which did not germinate after 46 days in the presence of 50, 100, 150, 200, and 300 mM NaCl, were germinated in pure water and recovery of germination capacity was determined as indicated in *Material and methods*. Different letters indicate statistically significant differences ($P < 0.05$) between treatments (by Tukey's test).

Discussion

Seed germination was delayed in the presence of NaCl and seawater and inhibited when NaCl concentration exceeded 100 mM and 5 % seawater dilution. Similar trends have been reported in other halophytes, which generally germinated better under reduced non-saline conditions. For instance, the germination of *Urochondra setulosa* was maximal in distilled water (100 %), but decreased to 20 % at 300 mM NaCl [5]. It has been reported that germination was substantially inhibited at 20 $g.l^{-1}$ NaCl in *Atriplex patula* [6]. Despite the depressive effect of salinity, halophytes are able to germinate at salt concentrations similar to that of sea water or even higher: in this way, seeds of *Triglochin maritima* germinated even at 400 mM NaCl [7], while *Kochia scoparia* germinated with a rate of 30 % at 1,000 mM NaCl [8]. The germination aptitude of *Spartina alterniflora* was preserved even at 1,027 mM, even with a very low rate of 8 % [9]. In addition, it was reported that the seeds of *Kochia americana* are able to germinate even at 1,712 mM NaCl [8].

Our results indicate that salt inhibited germination without damaging the seeds, which could recover their capacity to germinate when transferred to pure water. Hence, the main factor involved in the salt induced dormancy of *C. maritimum* seems to be the low water potential of the medium, as described for *Carpobrotus* [10], *Suaeda fruticosa*, *Triglochin maritima* [7] and *Aeluropus lagopoides* [11]. Our data support the assumption that salinity usually impairs germination by preventing imbibition [12].

The fact that salt pre-treatment did not affect seed viability could have a great significance for the ecophysiology of *C. maritimum*. Indeed, this finding suggests that *C. maritimum* would be able to constitute viable seeds bank when salinity levels are high, and that these seeds would germinate early in the spring, after salt leaching from the soil surface by the winter precipitations. This hypothesis should be checked with long salt treatments of seeds, to document the mortality kinetics of seeds in various conditions of salt and humidity.

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Biosaline Agriculture and Salinity Tolerance in Plants
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Growth performance and nutritional value of salt tolerant plants growing under saline environments

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Socio-economic effects of salinity

Salinity is a serious problem of agriculture worldwide, particularly in arid and semi-arid regions. Most of Pakistan is arid to semi-arid with low annual precipitation and 6.3 million hectares (mha) land is affected to varying degree of salinity [1]. As a result of this, heavy losses in crop yields and plant productivity have been recorded [2]. Lands with high salinity are not cultivated and are changing into wasteland, and farmers owning these lands are migrating towards cities or towns as a result of which population load on cities is increasing day by day and deficiencies in food, feed, fodder and industrial materials are being faced. So, there is an urgent need to utilize these lands for plant production [3].

Halophytes as livestock fodder

Halophytes having economic values can be grown on salt-affected wastelands, which may bring socio-economic gains. It was observed that farmers as well as livestock of salt-affected areas face great scarcity of food, fodder and feed. The farmers of these areas often arrange fodders for their livestock from other areas at very high costs. However, if these areas are utilized for developing pastures or rangelands with salt tolerant forage plants, good economic returns can be achieved. In Pakistan, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan, started work in the 1970s to identify the salt tolerant plants with economic values so that they may be utilized as food, fodder and industrial raw materials. Dozens of salt tolerant plants with economical value have been identified and introduced at two biosaline research stations. Some of these can be utilized as fodder; however, farmers are reluctant to use them as fodder due to their high salt content. It is necessary to have complete knowledge about the nutritive values of these plants, so that they can be recommended to the farmers for using them as fodder for their livestock.

The studies have been conducted at NIAB, Faisalabad, Pakistan, with the aim to work out the forage values and their chemical composition. On the basis of their utility as forage, these can be recommended for the livestock in saline areas and thereby salt-affected wastelands can be utilized for better economic returns. So, the present study was conducted with five halophyte forage plants, two were grasses and the other three were shrubs, i.e., *Leptochloa fusca*, *Sporobolus arabicus*, *Suaeda fruticosa*, *Kochia indica* and *Atriplex lentiformis*, respectively.

Cultivation of halophyte grasses, bushes and their chemical analysis

Studies were conducted at the Biosaline Research Station-II (BSRS-II) of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan, situated 40 km from Faisalabad, Pakistan. The salt concentration in ground water is 4,000–6,000 ppm and the water table varies from 2.1–3.0 m, while soil salinity ranges from 12–27 dS m^{-1}. The other characteristics of soil and water are summarized in Table 1. The site has an annual average temperature of 32^{o}C. The annual average precipitation and evaporation is 320 mm and 1,100 mm, respectively. The halophyte species viz, *Leptochloa fusca* (Kallar grass), (*Sporobolus* grass), *Suaeda fruticosa* (Lana), *Kochia indica* (Kochia) and *Atriplex lentiformis* (salt bush) were grown from stubbles and seeds in plots of 10×10 m, repeated four times; side by side naturally growing plants of these species were also selected. The irrigation water was underground brackish water with high salt (the characteristics of tubewell water are summarized in Table 2). When the plants were at the feeding stage, samples were collected from cultivated plots and naturally growing plants at BSRS-II. Their fresh and dry weights were recorded and to evaluate their nutritive values, samples were oven dried. The leaves and twigs were ground and used for subsequent chemical analyses. Crude protein (Nx6.25), fibers, total minerals and total carbohydrate were determined according to AOAC [4] procedures. The above ground plant material was digested according to

Table 1. Characteristics of soil of study site, BSRS-II Pacca Anna, NIAB, Faisalabad, Pakistan

Soil characteristics	**Range**
Soil texture	Sandy loam
Clay (%)	14 ± 1.4
Silt (%)	18 ± 1.5
Sand (%)	68 ± 3.1
EC (dS m^{-1})	12–27.24
Saturation Percentage	25.36–31.45
PH	7.82–8.92
Bulk density (g cm^{-3})	1.38–1.58
$CaCO_3$ (%)	12–23
$CaSO_4.2H_2O$ (%)	2.56–4.15

Table 2. Characteristics of Tube-well water at BSRS-II Pacca Anna, NIAB, Faisalabad, Pakistan

Characteristics	**Range**
EC (dS m^{-1})	4.97
PH	8.2
TSS	3878
SAR	40.5
SAR adj	101.25
RSC	21.60
Soluble ions (me L^{-1})	
Na^{+}	51.2
K^{+}	0.4
Ca^{2+} + Mg^{2+}	3.21
Cl^{-}	13.75
CO_3	1.5
HCO_3	21.75
SO_4	17.35

Wolf [5] and cations like Na^{+}, K^{+} and Ca^{2+} were estimated by flame photometer (FP7, Jenway, England) and from the same aliquot Mg was determined titrimetrically as described in US Salinity Lab Hand Book-60 and P using Barton's reagent [6]. The data were statistically analyzed [7].

Growth and yield of halophyte plants

The yield per plot is not shown because it is obvious that the plant with higher salt tolerance potential had higher yield and that was maximum in *Atriplex*, closely followed by *Suaeda fruticosa* and *Kochia indica* and minimum was in *Sporobolus*. The yield was higher in experimental plots than that of plants growing naturally. The pattern was almost similar in dry matter accumulation (Fig. 1a). As *Leptochloa fusca* and *Sporobolus grasses* excrete salt absorbed through the salt glands present in their leaves [8], so these species contained more water than other salt tolerant bushes [9]. The salt tolerant bushes contained more salts and less water, as a result they had more dry matter and total minerals. The palatability reports indicated that *Atriplex* and other salt bushes are not as palatable as *Sporobolus* and *Leptochloa fusca* and other grasses [9–11]. Similarly, Casson et al. [12] reported that higher dry matter in salt tolerant plants is due to high accumulation of salts, which is very clear from the present study, because the total mineral contents were very high in the salt tolerant bushes.

Mineral and nutrient values of halophytes

The total minerals were the maximum in *Atriplex lentiformis*, followed by *Suaeda fruticosa*, *Kochia indica*, *Leptochloa fusca* and *Sporo-bolus arabicus* (Fig. 1b), which

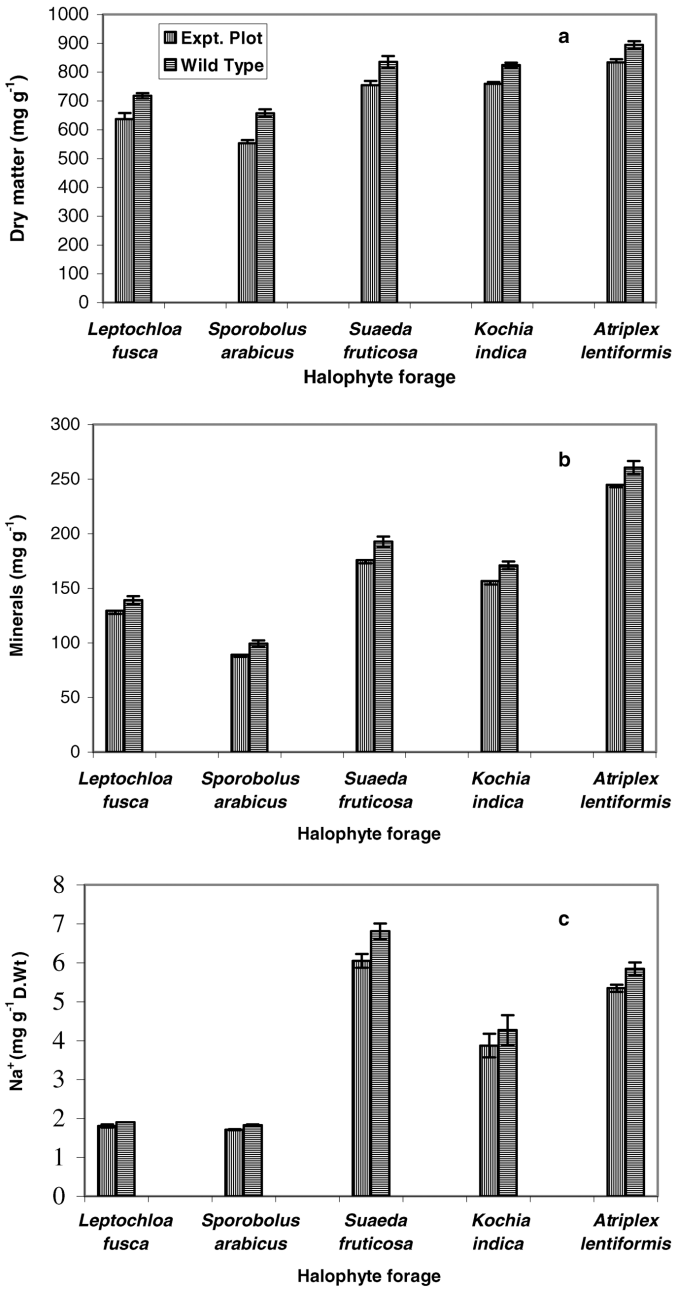

Figure 1. Growth performance and nutritional value of some halophyte forage species under saline environments (**a**) dry weight (**b**) total minerals (**c**) sodium contents

confirmed that maximum salts were accumulated in *Atriplex* and minimum in *Sporobolus*. On the basis of salt concentration, *Sporobolus* is categorized as the most palatable grass for livestock. Many reports indicated that the plants containing higher concentrations of salts are toxic for livestock and are responsible for different types of diseases and physiological disorders. The literature indicates that the animal fed on plants having higher concentrations of salts cause lesion and rashes in the stomach of the animals [13].

Chemical composition of the plants for ions like Na^+ (Fig. 1c), K^+ (Fig. 2a), Ca^{2+} (Fig. 2b) and Mg^{2+} (Fig. 2c) also varied significantly within different halophytic forage species. Maximum Na^+ was recorded in *Suaeda fruticosa* and minimum in *Sporobolus* and *Leptochloa fusca* (Fig. 1c). The literature has clearly indicated that the plants with higher concentrations of Na^+ are injurious for animal health and only the *Sporobolus* and *Leptochloa fusca* in experiment plots maintained Na^+ concentration which is near to critical limit for livestock. However, the naturally growing plants contained higher Na^+ concentrations near the critical limit necessary for livestock (Fig. 1c). So, *Sporobolus* and *Leptochloa* are more suitable as animal fodder than other salt tolerant plants. Many reports [9, 14] have suggested mixing of *Atriplex*, *Suaeda fruticosa* and *Kochia* in animal ration as a result of which Na^+ as well as other salt concentrations can be reduced in animal ration and can be made more palatable and digestible.

Potassium concentrations were higher in *Kochia*, closely followed by *Atriplex* and *Suaeda fruticosa*, while it was minimum in *Leptochloa* and *Sporobolus*. Although animals require higher K^+ concentration to maintain their optimum metabolic activities, in higher amounts it is toxic (Fig. 2a). The salt tolerant bushes contained higher amounts of K^+ than the critical limit; however, salt tolerant grasses contained K^+ up to the requirements of the animals. So these grasses can be recommended for direct grazing or feeding. However, for salt bushes, mixing with other plants is necessary.

Salt bushes also maintained relatively higher Ca^{2+} and Mg^{2+}. However, maximum Ca^{2+} was in *Atriplex*. *Suaeda fruticosa* contained the highest Mg^{2+} concentration (Figs. 2b and c). The grasses however, contained low amounts of these salts. This may be due to the excretion of salts through salt glands, which are absent in salt bushes [8]. The earlier work of NIAB scientists has demonstrated that these grasses have salt glands in their leaves to get rid of the excessive salt concentrations. This property of these plants therefore makes them more palatable and digestible. These grasses are already recommended to farmers for cultivation on salt-affected wasteland for livestock grazing. *Atriplex* and other salt bushes are also being utilized in the animal ration by mixing with other feed. Their sole use is not useful for livestock. Observations indicated that if the animals were only fed on salt bushes, they become weaker day by day and suffer from various metabolic or physiological disorders.

Maximum phosphorous was accumulated in *Suaeda fruticosa* and the minimum in *Sporobolus,* closely followed by *Leptochloa*. The salt bushes again contained more P than the critical limit for the livestock (Fig. 3a). P is a nutrient required in higher amounts for animals but its excessive amount may disturb the other metabolic functions of livestock. Reports have indicated [15, 16] that the salt grasses are useful for animals because they contain desirable amounts of P (Fig. 3a).

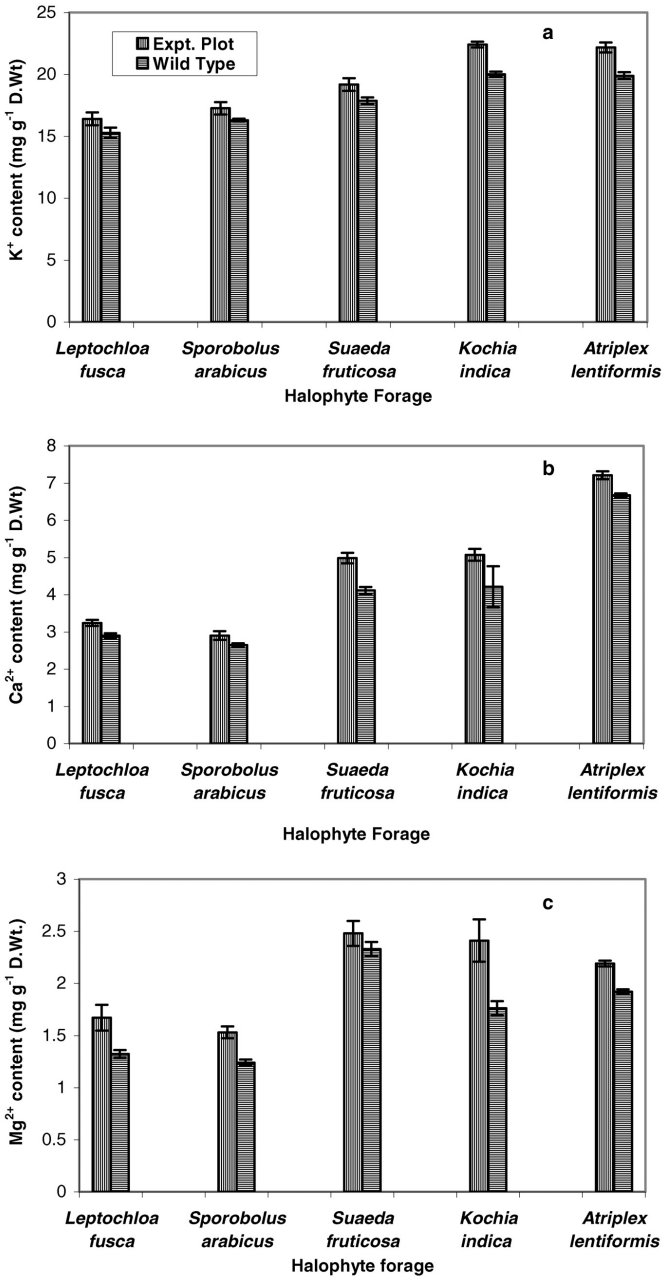

Figure 2. Nutritional value of some salt tolerant plants growing under saline environments (**a**) Potassium, (**b**) Calcium (**c**) Magnesium contents

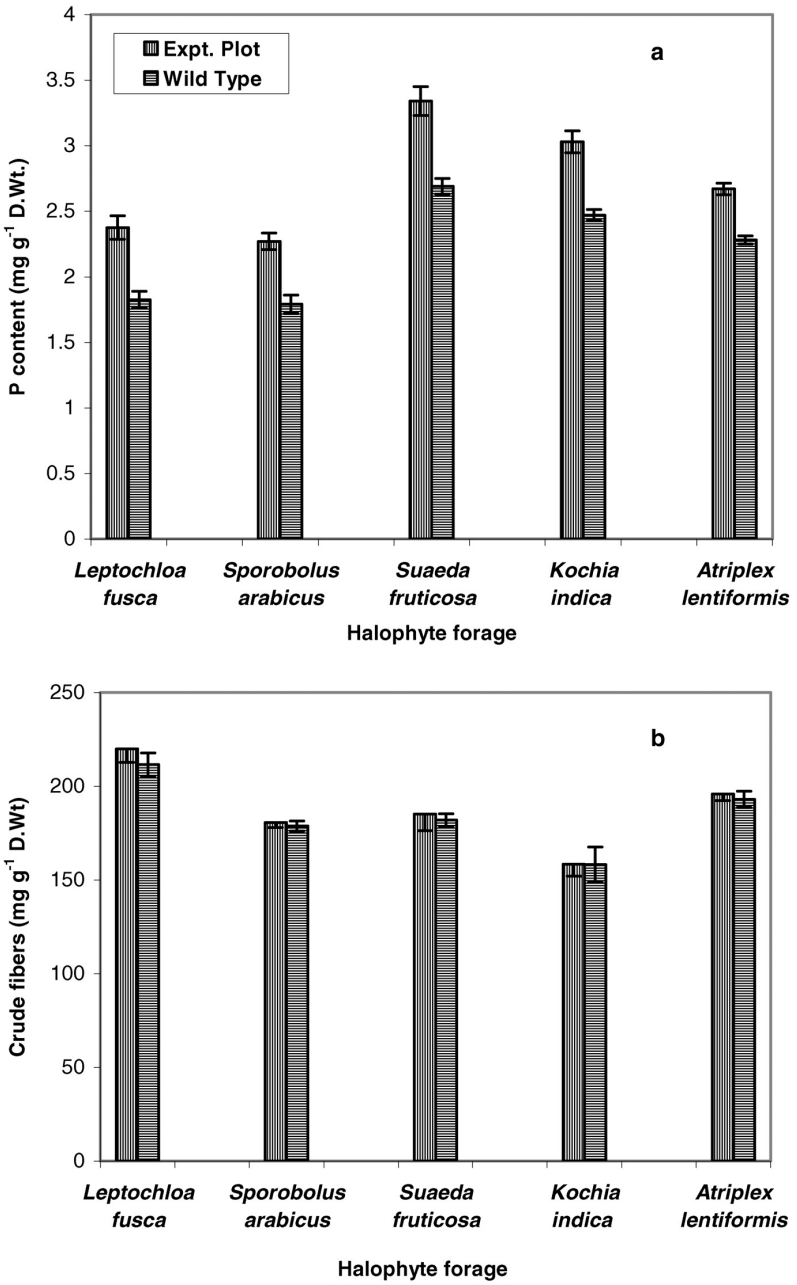

Figure 3. Nutritional value of some halophyte forage growing under saline environments (**a**) Phosphorus (**b**) Crude fiber contents

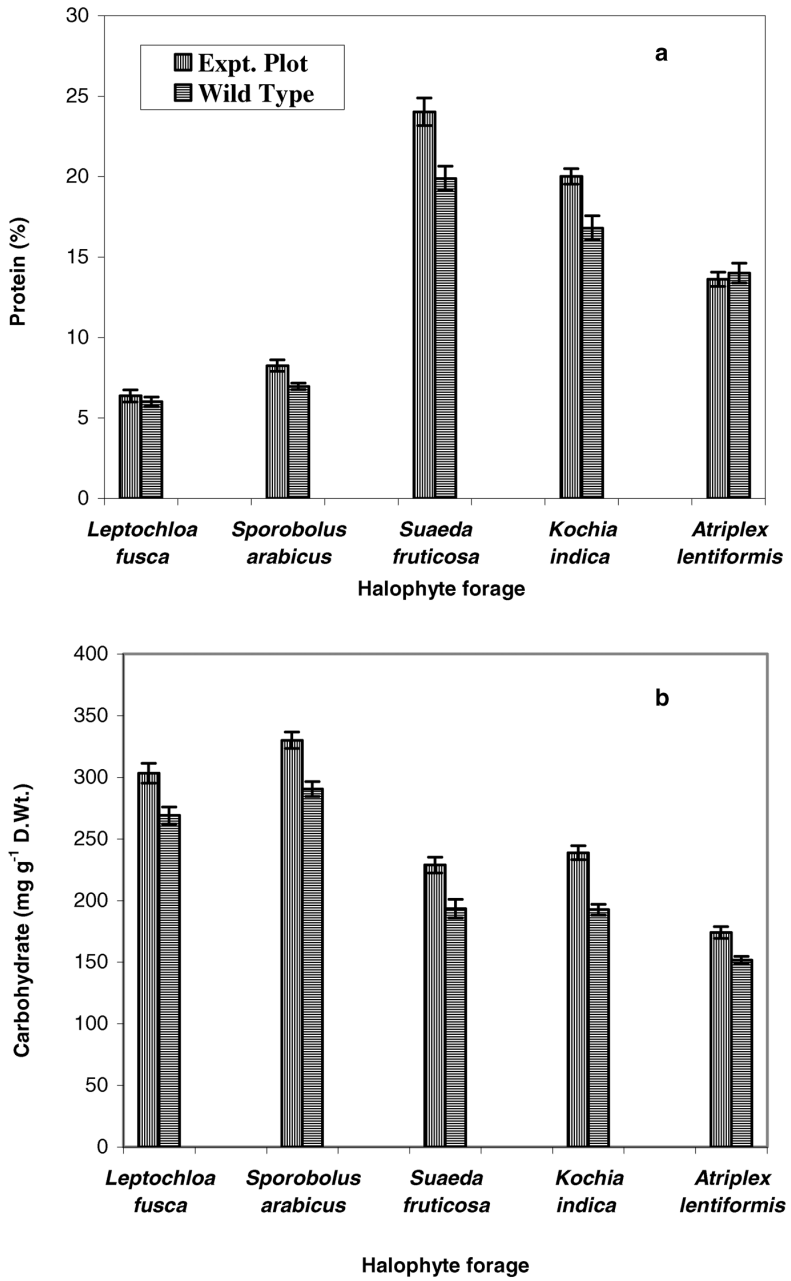

Figure 4. Nutritional value of some salt tolerant plants growing under saline environments (**a**) Protein (**b**) Carbohydrate contents

The results regarding *protein* contents of plants (Fig. 4a) also indicated that salt bushes had more protein than salt tolerant grasses, while reverse was the case for crude fibers (Fig. 3b). As regards the carbohydrates, they were the highest in *Sporobolus arabicus,* closely followed by *Leptochloa fusca* and minimum in *Atriplex lentiformis* (Fig. 4b). These findings also proved that *Sporobolus* and *Leptochloa* grasses are useful for animal health and contain nutrients up to the limit required by the livestock, while salt bushes contained more salts, which could create other metabolic disorders.

Recommendations

From the findings of the present investigation, it was also very clear that the cultivated plants contained less amounts of salts than ones growing naturally. This may be due to proper irrigation as a result of which they were able to contain reasonable amounts of water that caused dilution of salts, and plants became more suitable as animal fodder.

The palatability checked at NIAB [17] and at other places of the world also indicated that the *Atriplex*, *Suaeda fruticosa* and *Kochia indica* are not as suitable as the *Sporobolus* and *Leptochloa fusca*. The Animal Section of the Biological Chemistry Division of this institute has also conducted the digestibility studies of these species using the Nylon bag technique. It indicated that salt tolerant grasses had more digestibility values than salt bushes. So, on the basis of the above results *Sporobolus arabicus* and *Leptochloa fusca* are more useful and seem close to the normal fodder for livestock. The findings of the Animal Section of the Biological Chemistry Division of this institute has also showed that feeding of *Leptochloa* or *Sporobolus* grass had no adverse effects on health and reproduction of dwarf goats. Thus, they should be cultivated preferably on salt-affected wastelands to produce large amounts of fodder for livestock. The salt tolerant bushes should also be grown, but should be fed by mixing with other fodders containing lesser amount of salts.

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Comparative effect of NaCl and seawater on seed germination of *Suaeda salsa* and *Atriplex centralasiatica*

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Introduction

Seed germination in annual halophytes usually occurs when soil salinity levels are low and soil moisture is relatively high [1]. Optimum germination of halophyte seeds is often obtained under freshwater and inhibited by increasing salinity concentrations [2–4], but the ability to germinate at higher salinities is varied with species, for example *Salicornia herbacea* germinated at 1,700 mM NaCl [5], *Arthrocnemum macrostachyum* can germinate at 1,000 mM NaCl solution with 10 % germination percentage [6]. Some secreting halophytes could also germinate above seawater salinity [7–11]. Most secreting halophytes show germination at NaCl concentrations ranging from 0.34–0.52 M NaCl. Few of them have low salt tolerance during germination [12–14].

Seed of halophytes under natural conditions are subjected to saline stress dominated by NaCl. However, other chloride, sulfate and carbonate salts and their interaction play a significant role in affecting seed germination [1, 4]. There has been much research on halophyte seed germination in NaCl solutions. Such tests may not be relevant to the field conditions because in the field the soil solutions contains different cations (Na^+, Mg^{2+}, Ca^{2+}, K^+) and anions (Cl^-, ${SO_4}^{2-}$, HCO_3^- etc.), which compositions are similar to seawater. Different salt sources have different effects on seed germination [15]. Zia and Khan [16] reported that seawater inhibited seed germination of *Limonium stocksii* more than NaCl solutions, similar to Joshi et al. [17] reported for *Salvadora persica*. However, Duan et al. found that *Suaeda salsa* [18] and *Chenopodium glaucum* [19] germinated better in soil extracted solutions than in NaCl solutions, so did some combinations of salts have the similar effects on *Securigera securidaca* [20] and *Rhus chinensis* [21]. Those compared studies are still

limited on the relative tolerance of seawater and NaCl solution on seed germination of halophytes.

Recovery of germination responses in temperate halophytes has been demonstrated in *Salicornia europaea, Suaeda linearis* [22], *Spergularia marina* [9], *Suaeda depressa* [23], *Arthrocnemum australsicum, Triglochin striata, Suaeda australis, Juncus maritimus,* and *Casuarina glauca* [24]. Boorman [25, 26] and Woodell [7] also reported salt stimulation of seed germination following treatment with seawater for a number of saltmarsh species. Keiffer and Ungar [27] exposed seeds of five halophytes (*Atriplex prostrata, Hordeum jubatum, Salicornia europaea, Spergularia marina* and *Suaeda calceoliformis*) to an extended period of salinity treatments and determined their recovery responses when transferred to distilled water. They used Woodell [7] classification system and placed *Atriplex prostrata* seeds in the Type 1, *Hordeum jubatum* and *Spergularia marina* in the Type 2, and *Salicornia europaea* and *Suaeda calceoliformis* in the Type 3 category.

Suaeda salsa, a leaf succulent annual plant, and *Atriplex centralasiatica*, a secreting annual plant from the family Chenopodiaceae, are widely distributed in China and are quite common in coastal areas. Both species have the potential to be used as cash crops [28–30]. The present study investigates the effects of seawater and NaCl on the seeds germination of *S. salsa* and *A. centralasiatica* and compared the differential response of these two species.

Materials and methods

Seeds of *S. salsa* and *A. centralasiatica* were collected in October 2004 in the coastal saline soils of Haixing County, Hebei Province of China. Before storage they were surface sterilized using clorox (0.5 %) for 1 minute followed by thorough rinsing with distilled water and air-drying for a few days. After cleaning, seeds were stored in paper bags at room temperature with relative humidity 30–40 % in the laboratory and germination experiments were initiated in January 2005. Germination experiments were carried out in 10 cm diameter tight fitting plastic Petri dishes with two-layer wet filter paper. 10 mL of test solution were added in the Petri dishes to investigate the effect of salinity on seeds germination of *S. salsa* and *A. centralasiatica*. Germination was carried out in different dilutions (0, 5, 10, 20, 30, 40, 50 dS m^{-1}) of NaCl and seawater separately. The electrical conductivity for both salt solutions was maintained at the desired level with the help of a conductivity meter. Four replicates of 40 seeds each were used for all treatments. Seeds with visible radicle were considered to have germinated.

A 24 h cycle was used where day temperature (25° C) coincided with 12 h light period (cool white fluorescent lamps, 25 uM $m^{-2}.s^{-1}$ 400–750 nm) and night temperature (15° C) coincided with the 12 h dark period. Percent germination was recorded every day for 15 days for all experiments. Un-germinated seeds from the salinity treatments were transferred to distilled water to study the recovery of germination, which was recorded each day for 7 days. The recovery percentage was determined by the following formula: (a–b)/(c–b) $\times$ 100; where a = the total number of seeds

germinated after being transferred to distilled water, b = the total number of seeds germinated in saline solution and c = the total number of seeds. The rate of germination was estimated by using modified Timson's index of germination velocity: $\sum G/t$ – where G is the percentage of seed germination at one day interval, and t is the total germination period [2, 31]. The maximum number in our system could be 100. The higher value the more rapid the germination.

Data were analyzed using SPSS for window release 11.0. LSD test was used ($p < 0.05$) to determine significant differences among salinity treatments and species in seawater and NaCl separately.

Results

Seed germination in both *S. salsa* and *A. centralasiatica* remained unaffected up to 20 $dS.m^{-1}$ NaCl and seawater treatments (Fig. 1). A further increase in salinity inhibited germination in both species; however, only 8 % of seeds germinated at highest salinity treatments in the case of *A. centralasiatica* in comparison to 56 % germination in *S. salsa* in NaCl solution. NaCl inhibited germination more in comparison to seawater solutions (Fig. 1).

In non-saline control, highest germination was recorded at 2 days in *S. salsa* and 6 days in *A. centralasiatica* (Fig. 2). Increase of salinity delayed the germination and at EC 50 $dS.m^{-1}$ salinity level first seed germinated after 9 days in NaCl solution and only 11 % seeds germinated in seawater solution in *A. centralasiatica*. While in *S. salsa* 46 % and 58 % seeds germinated in NaCl and seawater solutions, respectively.

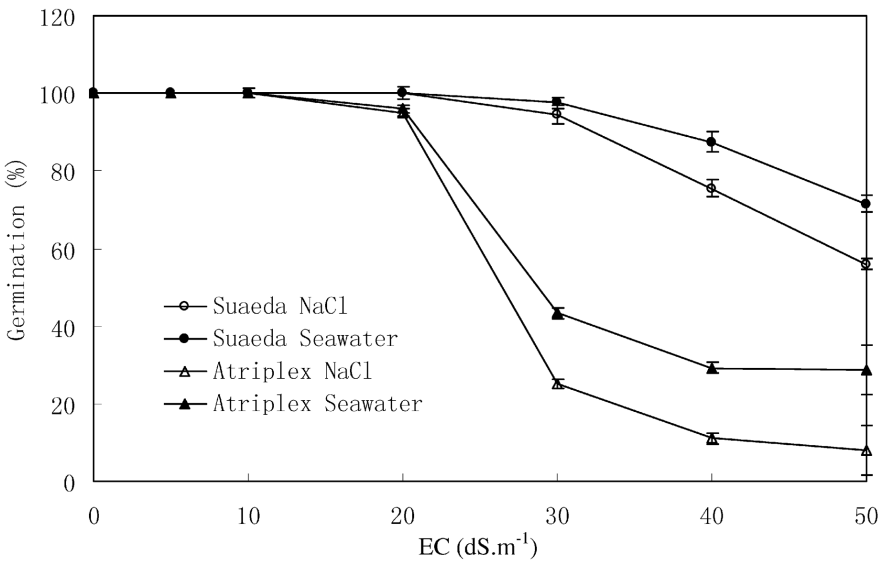

Figure 1. Mean final germination percentage of *Suaeda salsa* and *Atriplex centralasiatica* in various NaCl and seawater solutions. Bars represent means (± s.e., n=4).

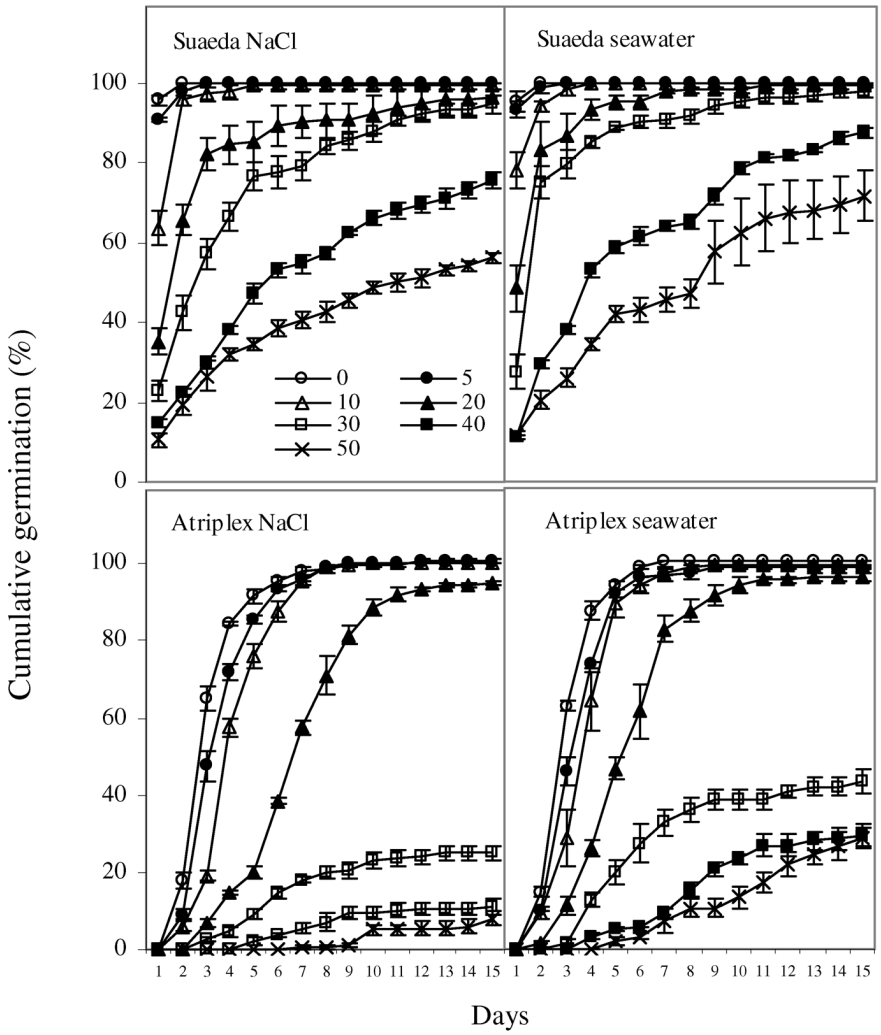

Figure 2. Cumulative mean germination percentage of *Suaeda salsa* and *Atriplex centralasiatica* over time in various NaCl and seawater concentrations. Bars indicate s.e. of means (n=4).

Rate of germination was highest in non-saline controls, and higher in *S. salsa* seeds than in *A. centralasiatica* seeds in all treatments and decreased with the increase of salinity (Fig. 3). Seeds of both species germinated slowly in NaCl solutions than in seawater solutions.

Un-germinated seeds from both NaCl and seawater solutions when transferred to distilled water recovered completely, but higher salinities affected the recovery of *A. centralasiatica*, and greater in seawater solutions than in NaCl solutions, for

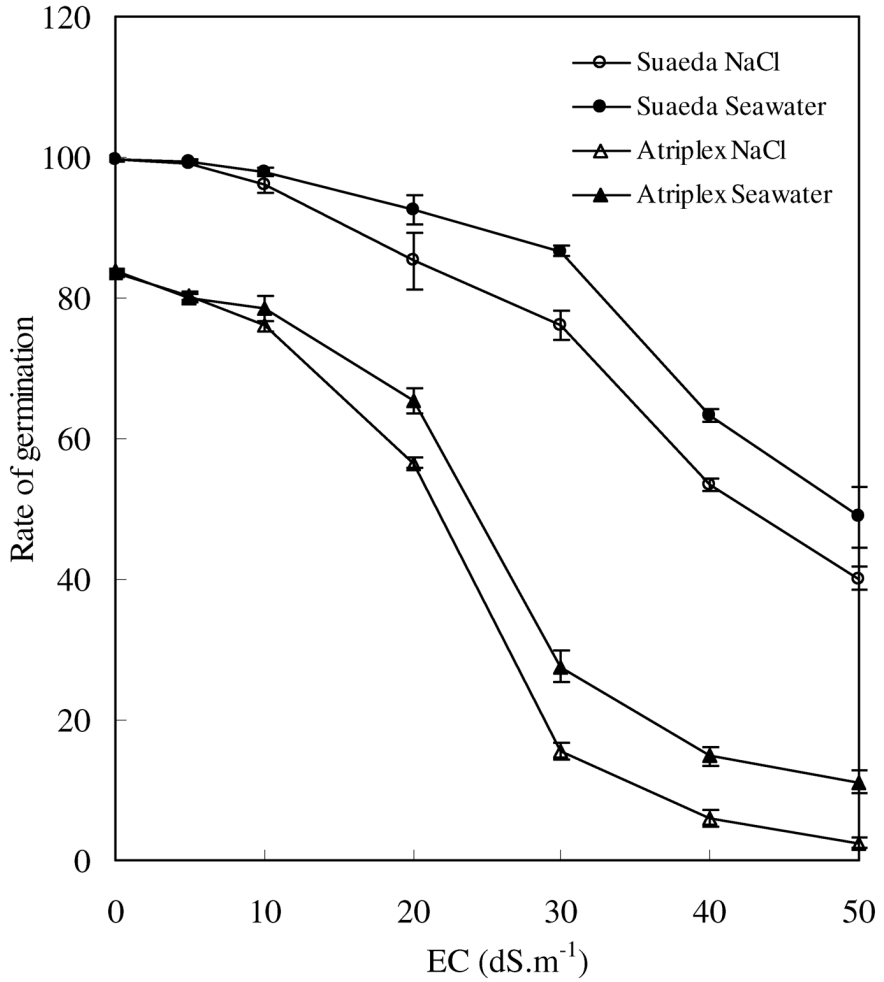

Figure 3. Rate of germination of *Suaeda salsa* and *Atriplex centralasiatica* seeds under various NaCl and seawater concentrations. Bars represent means (±s.e., n=4).

example, un-germinated seeds from 50 $dS.m^{-1}$, *S. salsa* can fully recovered at 1 day and 97 % in NaCl and 82 % in seawater of *A. centralasiatica* recovered at 7 days (Fig. 4).

Discussion

The vegetation along the coast of Northern China is dominated by a stem succulent halophyte *Suaeda salsa* and a secreting halophyte *Atriplex centralasiatica*. Coastal communities are usually mono-specific and their distribution is controlled by inundation gradient and their frequency. *S. salsa* occupy low marsh habitat with high

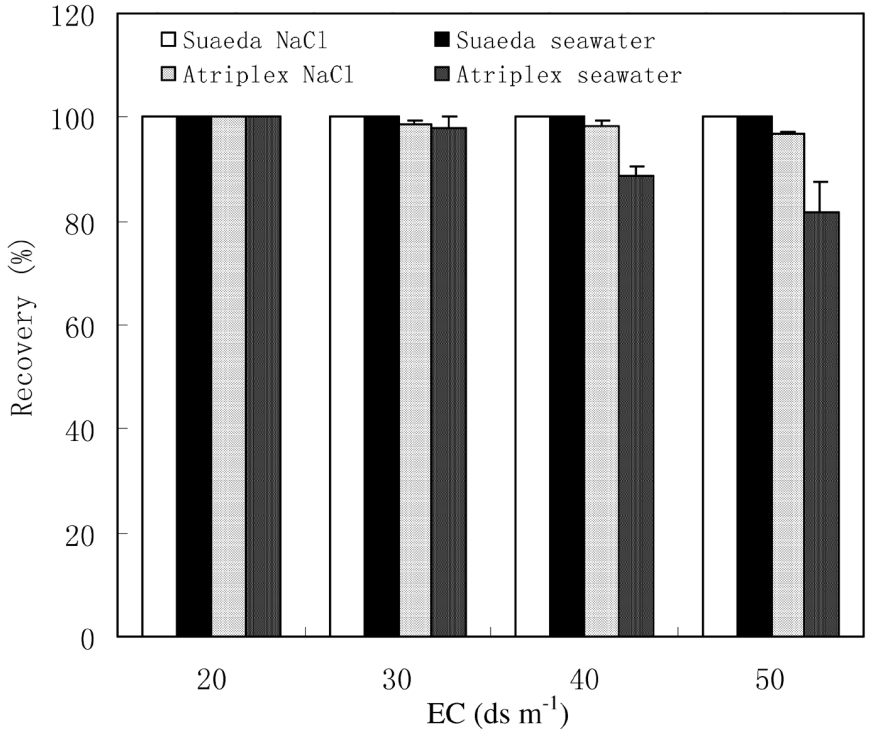

Figure 4. Mean recovery percent germination for *Suaeda salsa* and *Atriplex centrlasiatica* in distilled water under the treatments of various NaCl and seawater concentrations. Bars represent means (±s.e., n=4).

salinity and high moisture, while *A. centralasiatica* occupy a higher ground with infrequent inundation and low salinity and moisture conditions. This difference in ecological habitat has conferred varied ecological strategies to both species particularly at germination level. Seeds of *S. salsa* are highly salt tolerant and could germinate at seawater salinity, while few seeds of *A. centralasiatica* could germinate at that level. Halophytes vary in their ability to tolerate salinity at different stages of the life cycle. They are usually very highly salt tolerant while stored in the soil; however, their tolerance decreases at the germination and in most cases it increases again at the growth stage [4]. Seeds of leaf succulent species are highly tolerant to salinity [4]. Salinity plays an important role in determining the germination and survival of *Suaeda* spp [32] and it can tolerate high salinity during germination [32, 33]. The limit of salt tolerance of different species of *Suaeda* varies from 400–800 mM NaCl [22, 23, 34–36]. Secreting halophytes that could germinate above seawater salinity are few [7–11]. Most secreting halophytes show germination at NaCl concentrations ranging from 0.34–0.52 M NaCl [4, 37] while few of them have low salt tolerance during germination [12–14].

NaCl inhibited more seed germination in comparison to seawater solution both in *S. salsa* and *A. centralasiatica*. There is little information available on the effect of seawater on the germination of halophytes [7, 16, 17, 38, 39] and on the relative tolerance of seawater and NaCl solutions during seed germination [16, 17, 40]. Some results of combined salts effects on seed germination showed that the inhibitory effect of single salts can be considerably alleviated in natural soil systems by synergistic interactions between salts [18–21]. The increased seed germination in seawater may follow the same rule as combined salt effect because in seawater a lot of ions were included. However, seeds of *Limonium stocksii* [16] and *Salvadora persica* [17] were inhibited more by seawater; they also attributed this effect to seawater composition. This needs the further investigation.

Seeds of halophytes have the unique property of surviving extremely high salinity during the storage in the seed bank [41] and they germinate readily when soil salinity is reduced. However, recovery responses vary from one species to the other and against the level of salinity they are exposed to [27]. Seeds of *S. salsa* recovered completely when exposed to higher concentrations of NaCl and sea salt. Recovery of *A. centralasiatica* was also very high, except at 50 $dS.m^{-1}$ where 12–20 % of seeds failed to recover. A substantial recovery from germination occurred at the NaCl concentrations up to 600 mM NaCl in *Halogeton glomeratus* [42], *Sarcobatus vermiculatus* [43], *Suaeda moquinii* [36] and *Triglochin maritima* [44].

Suaeda salsa and *A. centralasiatica* are highly salt tolerant halophyte species where *S. salsa* is more salt tolerant than *A. centralasiatica*. Seeds of both species could germinate at seawater level but more germination was recorded in the seeds of *S. salsa*. Seed germination of *S. salsa* was more rapid in comparison to *A. centralasiatica*. Both species have a high ability to survive under extreme conditions; the un-germinated seeds can recover completely when the salinity stress was removed. Seed germination was inhibited more by NaCl than seawater. The literature suggests that the germination of halophyte seeds have differential response to seawater and NaCl. Some reports indicate that seawater inhibits more seed germination, while others believe that it is NaCl. Further studies will be carried out to understand the difference between NaCl and seawater effects on germination.

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Biosaline Agriculture and Salinity Tolerance in Plants
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Salt effect on growth, photosynthesis, seed yield and oil composition of the potential crop halophyte *Cakile maritima*

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Introduction

Salinity is an extending environmental issue which compromises the long-term sustainability of agriculture, especially in the coastal semi-arid areas [1]. This is the case in Tunisia, where the semi-arid Mediterranean climate prevails (mean annual precipitation of 200–700 mm). Subsequently, around 10 % of the whole territory would be salt-affected [2]. Halophytes have evolved a wide range of attributes (morphological, physiological and biochemical) allowing them to tolerate the presence of salt in the medium [3]. Besides, several studies suggest that these plants are potentially useful for ecological and economical purposes [4].

Intracellular salt flux control is one of the major salt tolerance determinants, involving salt exclusion and/or compartmentation. P and V H^+-ATPases (respectively localized at the plasma membrane and tonoplast) provide energy for Na^+/H^+ antiporters, thus allowing sodium active transport away from the cytoplasm [5]. The bi-directional transport of sodium insures ion homeostasis, cell turgor, as well as the metabolic functioning [6]. On the other hand, impairment of the photosynthetic activity greatly accounts for growth restriction of non-halophytes under salinity [7]. Depressive effects of salinity are thought to arise from stomatal and/or non stomatal limitations (i.e., stomatal closure and/or damage to Calvin cycle enzymes) [8].

Cakile maritima (*Brassicaceae*) is an annual fleshy halophyte which colonizes the sandy beaches of the Tunisian littoral. This study aims to characterize the plant response to long-term salt treatments (0–500 mM NaCl), using physiological (growth, water status, mineral nutrition) and biochemical (H^+-ATPase activity and photosynthetic capacity) criteria. Changes in seed yield and seed oil characteristics under salinity are also assessed.

Material and methods

Mature seeds of *C. maritima* were harvested on sandy beaches of Raoued (20 km to the north of Tunis). Seedlings were grown in pots filled with inert sand in a glasshouse (16 h/8 h light/dark regime; 60 % relative humidity; 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic active radiation – PAR; $22 \pm 1^{\circ}$ C temperature). Irrigation was performed with a Long Ashton nutrient solution [9]. Four week-old plants were progressively submitted to increasing salinities (0–500 mM NaCl) for 6 weeks. The same experience was repeated until the complete maturation of seeds. At the final harvest, physiological parameters were determined (i.e., dry weight, leaf number and area, leaf succulence ratio, leaf ion status). Yield components assessed were seed yield, seed mass, seed viability, and seed oil content.

Expanded leaves situated on the fifth node from the shoot top were used for photosynthetic and H^+-ATPase activity measurements. Leaf gas exchanges were measured with a portable photosynthesis system (LCi, ADC Bioscientific Ltd., UK) at 2,500 $\mu mol.m^{-2}.s^{-1}$ PAR (saturating light). Ribulose-biphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39) activity was spectrophotometrically assayed (λ = 340 nm) [10]. Vacuolar and plasma membrane (respectively, V and P) H^+-ATPase activities were assayed on isolated chloroplasts, using [$\gamma - ^{32}$P] ATP (1 MBq) (Hartmann Analytik, Braunschweig, Germany) [11]. Seed total lipids were extracted [12] and triacylglycerols (TAG) were separated by thin layer chromatography (TLC), using silica gel plates (Merck G 60) [13]. Fatty acid methyl esters were quantified by adding heptadecanoic acid (17:0) as an internal standard. Results are the means of three samples. A one way ANOVA was achieved to compare the mean values, using the SPSS statistical program ($P < 0.05$). In case of significant differences, Duncan *post hoc* tests were performed.

Results and discussion

Moderate salinities (50–100 mM NaCl) were optimal for the plant growth, since improving whole plant dry weight (+24 % at 100 mM NaCl) (Fig. 1A). No significant growth decrease occurred in the 200–300 mM NaCl range (ca. 90 % of control values), and the plant was able to survive, even at a salinity close to that of seawater (500 mM NaCl). These data corroborate previous investigations on other halophytes, showing sub-optimal growth in mediums lacking salt [14, 15]]. Leaves largely accounted for the plant response pattern, since their dry weight and number were significantly stimulated at optimal salinities (50–100 mM NaCl) (Figs 1A and 1B). Leaf water status, evaluated by leaf succulence ratio, was significantly enhanced by salt treatments (Fig. 2A), and remained higher than the control values, even at 500 mM NaCl. The improvement of leaf hydration under salinity was concomitant with the accumulation of high amounts of Na^+, and at a lesser content of Cl^- (Fig. 2B) (1.8 and 3.8 $mmol.g^{-1}$ DW, respectively). Since salt treatment did not impair leaf hydration, most of Na^+ ions transported in leaves might have been removed from the leaf apoplast and efficiently compartmentalized by cells for water retention. Salinity restricted the plant nutrient uptake, leading to a significant decrease in leaf K^+

contents (Fig. 2B). The same tendency was observed for Ca^{2+} and Mg^{2+} (data not shown). The salt-induced reduction of growth could be a consequence of nutritional imbalance. Moreover, despite Na^{+} is a cheap osmoticum for halophytes, an excess of this ion over K^{+} can inhibit several metabolic processes.

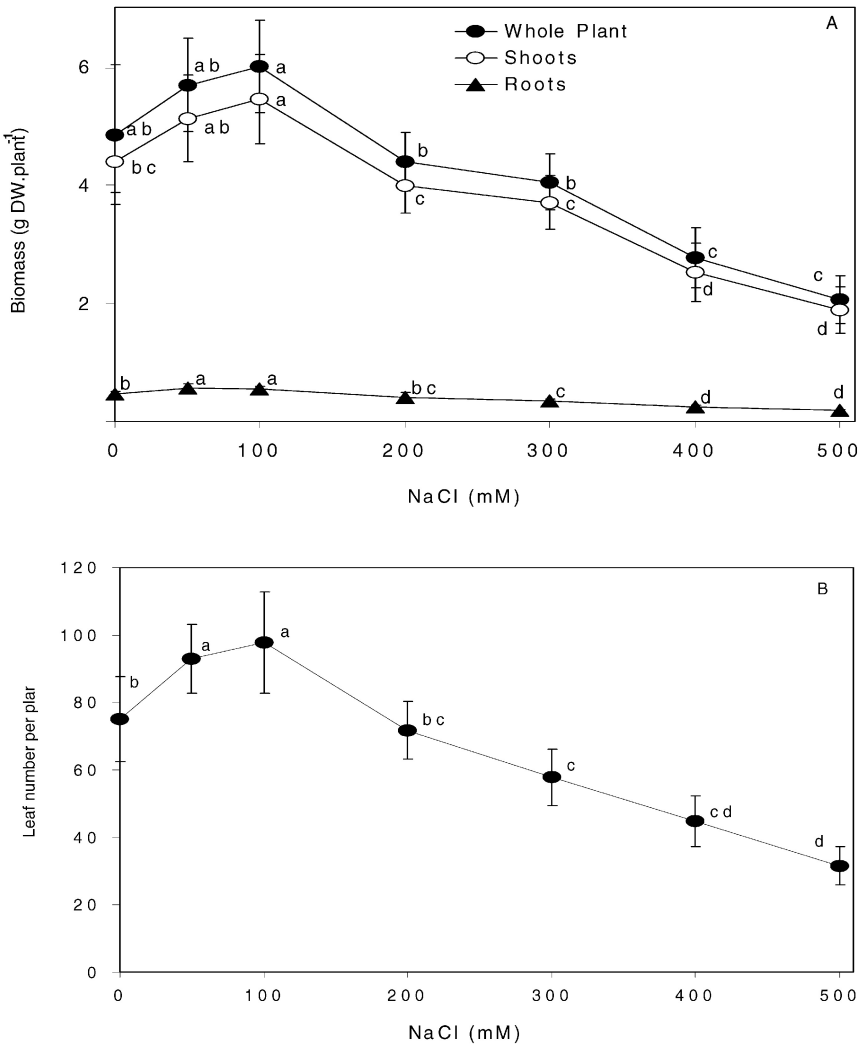

Figure 1. Effect of NaCl on growth of *C. maritima*. (**A**) Biomass production of the whole plant and the different organs. (**B**) Leaf number per plant. Means of 18 replicates ± SE. (Values within each salt treatment marked with at least one same letter are not significantly different at $P < 0.05$.)

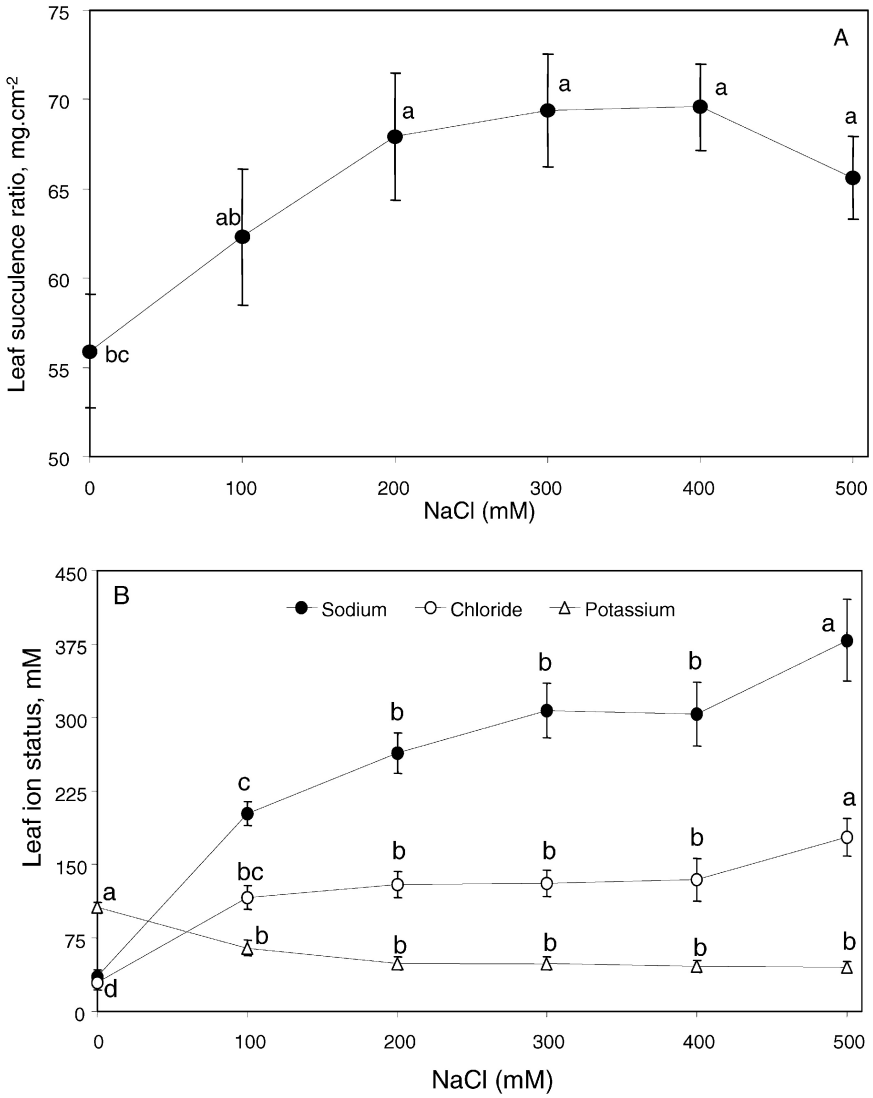

Figure 2. Effect of NaCl on leaf water status and mineral nutrition of *C. maritima*. (**A**) Leaf succulence ratio. (**B**) Leaf ion contents. Means of 18 replicates ± SE. (Values within each salt treatment marked with at least one same letter are not significantly different at $P < 0.05$.)

Combining the results relative to the leaf water status and salt accumulation of salt-treated *C. maritima* provide indirect evidence for the existence of salt compartmentation mechanisms within leaf cells (i.e., inclusive strategy). This assumption was confirmed by (i) the strong stimulation of V H^+-ATPase activity up to 300 mM NaCl (+80 %/control) (Fig. 3A) and (*ii*) the absence of anatomical structures

responsible for salt exclusion at the leaf surface. NaCl concentrations in the 300–500 mM range significantly promoted P H^+-ATPase activity, suggesting that the exclusive pattern may take place at high salinities (Fig. 3B). Keeping sodium and chloride away from cytosol (using inclusive strategy) is of vital importance for dicotyledonous halophytes lacking morphological structures of salt excretion at their leaf surface [16]. Owing to their catalyzer role, proton pumps enable both vacuolar and plasma membrane antiporter functioning, and play therefore, a major role in salt tolerance [17]. In addition, the overexpression of Na^+/H^+ antiporters plants has been reported to improve the performance of several species in saline conditions [18].

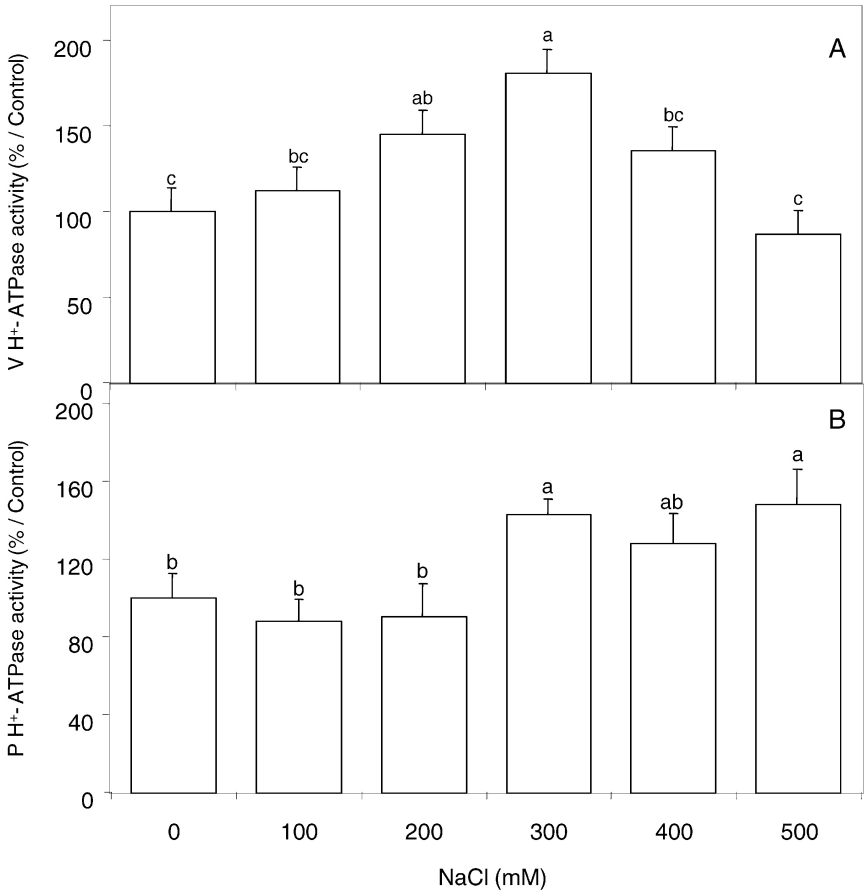

Figure 3. Effect of NaCl on H^+-ATPase activity (%/Control) of *C. maritima*. (**A**) Changes in vacuolar V H^+-ATPase activity. (**B**) Changes in plasma membrane P H^+-ATPAse activity. Means of three replicates $\pm$ SE. (Values within each salt treatment marked with at least one same letter are not significantly different at $P < 0.05$.)

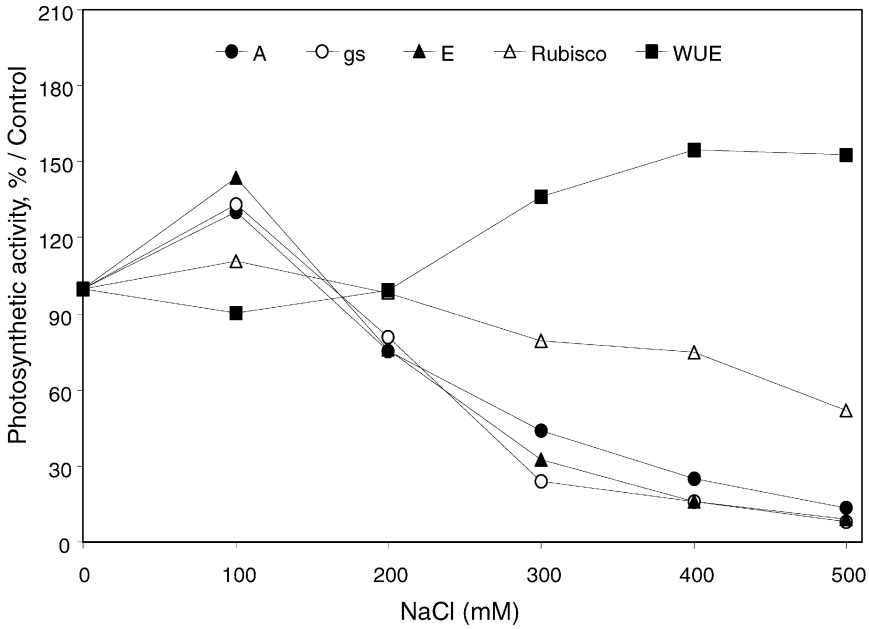

Figure 4. Effect of NaCl on photosynthetic activity of *C. maritima*. Results of gas exchanges are the means of 10 replicates. Results of Rubisco activity are the means of three replicates.

Both stomatal and non stomatal components of photosynthesis were improved at optimal salinity for growth. CO_2 assimilation rate (A), stomatal conductance (g_s), and transpiration rate (E) were 30–40 % higher at 100 mM NaCl, while specific activity of Rubisco was augmented by ca. 10 % (Fig. 4). Supra-optimal salinities impaired photosynthetic activity, but this depressive effect was more pronounced on stomatal conductance than on enzyme activity (respectively 15 % and 75 % of the control values at 400 mM NaCl). Former studies showed that stomatal limitation accounted for the reduction of photosynthesis in salt-treated plants [8]. In *C. maritima*, stomata closure was associated with reduced transpiration rate (E), leading to higher water-use efficiency (+ ca. 50 % at 500 mM NaCl). No salt-induced shift in the photosynthetic pathway (C_3 to C_4) was observed, since phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.39) activity remained lower than Rubisco one, irrespective of salt treatment (data not shown).

Optimal salinities for growth and photosynthesis promoted significantly seed production (+50 % in the 50–100 mM NaCl range) (Fig. 5A). This parameter was more affected than plant growth at high salinities (respectively 21 % and 84 % of the control values at 300 mM NaCl), likely resulting from a reduction of flower production and/or a decrease of their fertility [19]. The mean mass of individual seed decreased significantly in the presence of salt in *C. maritima* (Fig. 5A), indicating that assimilate allocation to seeds was more restricted by salt than seed initiation. Seeds harvested from plants exposed to mild salinities (50–200 mM NaCl) displayed

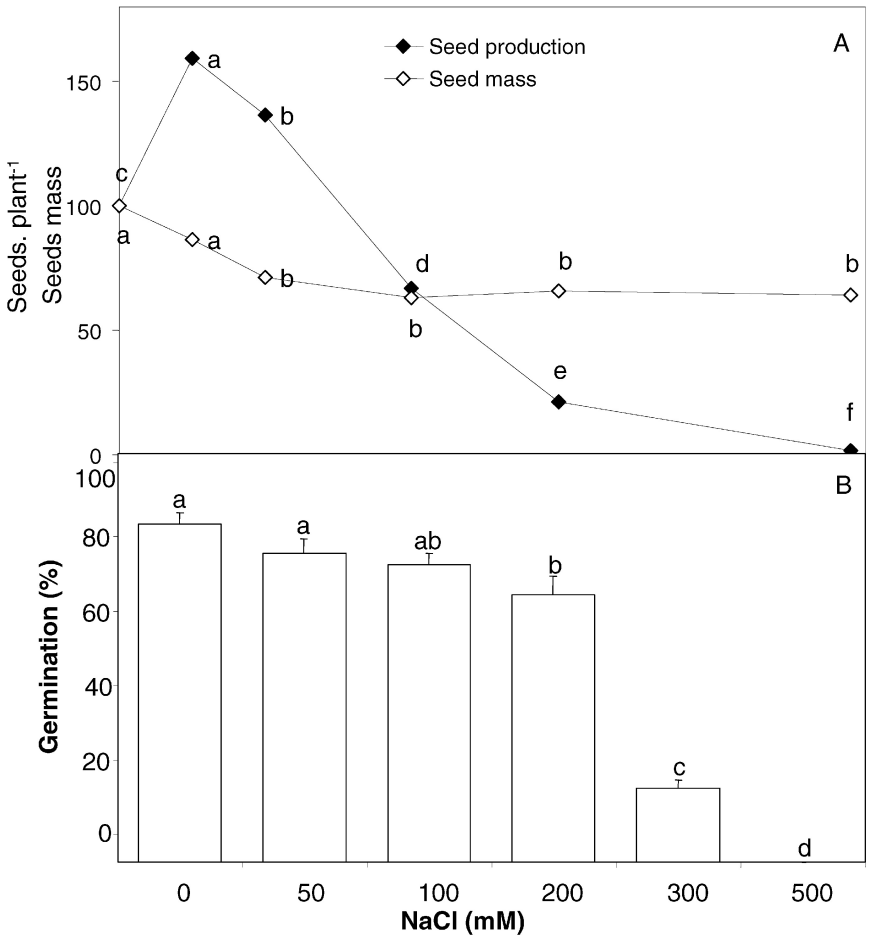

Figure 5. Effect of NaCl on the reproductive capacity of *C. maritima*. (**A**) Seed production per plant (means of 12 replicates) and individual seed mass (mg) (means of 300 replicates), expressed as % of the control. (**B**) Germination capacity (%) in distilled water of seeds harvested from plants exposed to increasing salinities. Means of three replicates ± SE. (Values within each salt treatment marked with at least one same letter are not significantly different at $P < 0.05$.)

high germination rates (up to 80 %), contrasting with those produced under high salt levels (Fig. 5B). Increasing salinities led to both quantitative and qualitative changes in the seed oil characteristics. Seed oil content (on a dry weight basis) was positively correlated with the medium salinity (respectively 30 % and 28 % at 100 mM and 500 mM NaCl). Seed oil content seemed also to be unaffected by salinity in the oleaginous halophyte *Lesquerella fendleri* [20], while decreasing in sunflower [21]. Erucic acid (22:1) level increased markedly, reaching 26 % at 500 mM NaCl (two-fold higher than control values). This trend was concomitant with a significant decrease in oleic

acid (18:1) level (ca. 45 % of the control value at 500 mM NaCl). In our conditions, higher erucic acid in salt treated *C. maritima* was associated with increased 22:1/18:1 ratio (0.39 and 1.36, respectively for the control and 500 mM NaCl plants), likely mediating elongases, which are known to catalyze the formation of long fatty acids (such as erucic acid), using oleic acid as initial substrate [22].

In summary, the present study shows that moderate salinities are required by *C. maritima* to express maximal growth and seed production potentialities, in relation with the concomitant involvement of several processes at different levels. Further field experiments are necessary to confirm the economic potential of this promising halophyte.

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Biosaline Agriculture and Salinity Tolerance in Plants
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Effect of nitrogen deficiency, salinity and drought on proline metabolism in *Sesuvium portulacastrum*

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Introduction

Drought and high salinity are responsible for large decreases in crop productivity all over the world [1]. These losses of crop yield result from limitations of plant development through excessive ion accumulation, water deficit and mineral deficiencies [2]. Under these prevalent stresses, tolerant plants adopt various strategies with a wide range of biochemical to physiological and morphological adaptations [3]. Morphological ones include modifications in growth and allocation of assimilates towards roots for an efficient exploitation of soil nutrients [4]. The physiological strategy is represented by a higher selectivity for K^+ over Na^+ [5], an increase in K^+-use efficiency [6], and the synthesis of organic osmolytes, with low molecular weight, for osmo-protection [7]. These osmolytes are sugars, polyols, amino acids, tertiary and quarternary ammonium, and tertiary sulphonium compounds [8].

The accumulation of compatible solutes induces a decrease in the water potential and allows additional water to be taken up from the environment [9]. In our study we focused on proline accumulation. Proline is commonly referred to as compatible solute in many eubacteria, algae, and higher plants [10]. The accumulation of proline is due primarily to *de novo* synthesis [11, 12], secondary to a reduced rate of catabolism [11], and finally to specific transport systems that distribute proline to the locations of need [13]. Two possible pathways of proline synthesis have been shown in plants. One is using glutamate (Glu) and the second is using ornithine (Orn) as a precursor [10]. Proline degradation in plants takes place in mitochondria and is catalysed by proline dehydrogenase (ProDH), also named proline oxidase [14]. Proline degradation has been shown to be inhibited under water and salt stresses. Both a decrease in ProDH mRNA level [15] and ProDH activity [16] result in proline accumulation.

In the present study, we investigated *S. portulacastrum* response to the availability of nitrogen in the presence of NaCl or under water stress. We measured δ-OAT and

ProDH activities to evaluate the relationship between enzyme activities and proline concentrations in leaves.

Material and methods

Culture

3 cm long stem segments with one node and two opposite leaves were taken from mother plants of *Sesuvium portulacastrum*, disinfected for 5 min in saturated calcium hypochlorite solution, and rinsed abundantly with distilled water. They were then placed for 7 days in an aerated Hewitt [17] solution diluted 10 times, supplemented with Fe K EDTA [18] and micronutrients [19]. Rhizogenesis took place during this week.

To determine the response of *S. portulacastrum* to the availability of nitrogen in presence of 400 mM NaCl (increased by 100 mM daily to reach the maximum salinity levels [20]), plants were submitted to a discontinuous nitrogen supply. After 35 days of pretreatment, an initial harvest was achieved. Plants were divided in two lots: in the first one, plants were cultivated on a complete nitrogen mode, 14.4 mM (+N), in the second one plants were subjected to limiting nitrogen supply 0.28 mM (–N). After 5 weeks of treatment, one lot of deficient plants was transferred on a complete nitrogen mode ($\pm$ N plants). Shoots and roots were weekly harvested during 70 days.

The second experiment aimed at determining the response of this halophyte to water deficit. Plants cultivated individually in pot filled with limono-sandy soil, were divided in two lots: the first one was irrigated with tap water at 100 % field capacity (FC) corresponding to control plants, and the second one at only 25 % FC (dehydrated plants). After 16 days of treatment, one lot of dehydrated plants was rewatered at 100 % FC. All cultures were carried out in a greenhouse with a 14 h photoperiod. Mean temperature and relative humidity were respectively $30 \pm 5^\circ$ C, 55 ± 5 % day and $16 \pm 2^\circ$ C, 90 ± 5 % night. Shoots and roots were harvested every 4 days during 40 days.

Plant analysis and enzymatic assay

During the harvests, shoot and root dry weights were measured, after desiccation for 48 h at 60° C. Reduced nitrogen was measured according to the Kjeldahl method. Proline was extracted and estimated by the method suggested by Bates et al. [21]. Frozen leaves (three samples of approximately 1 g FW per treatment) were grounded to a fine powder in a chilled mortar and pestle in the presence of PVP (0.2 g/g FW), and then homogenized in an appropriate extraction buffer. Ratios for buffer volume/g FW were 2:1. All operations were carried out at 4° C. Extraction buffer of δ-OAT (EC 2.6.1.13) consisted of 100 mM K-Pi buffer (pH 7.9), 1 mM EDTA, 15 % glycerol and 10 mM 2-mercaptoethanol. The extract was centrifuged at $15,000 \times g$ for 15 min. Extraction buffer used for ProDH (EC 1.5.99.8) was 50 mM Tris-HCl buffer (pH 7.4) containing 7 mM $MgCl_2$, 0.6 M KCl, 3 mM EDTA and 1 mM DTT. The extract was centrifuged at $39,000 \times g$ for 20 min [22]. δ-OAT activity was assayed

with ninhydrin according to Kim et al. [23]. Enzyme activity was expressed as μ moles of pyrroline 5-carboxylate formed per hour and per mg of proteins. ProDH was assayed by following the $NADP^+$ reduction at 340 nm in a 0.15 M Na_2CO_3-HCl buffer (pH 10.3) containing 15 mM L-proline and 1.5 mM $NADP^+$ [24].

Results

Changes in plant response to osmotic stress in relation to nitrogen availability

Plants subjected to limited nitrogen supply showed an inhibition of their growth, which amounted to approximately 70 % of control (at the end of the treatment) (Fig. 1). The difference between these two treatments appeared only after 35 days. The transfer of the plants from (–N) to (+N) medium restored the growth (plants -+N). In plants with appropriate nitrogen nutrition, the leaf proline concentration regularly increased to a maximum value at 28 days. During the four last weeks of treatment, proline concentration decreased significantly. In (–N) plants, proline concentration remained low representing only 50 % of the control at the end of treatment. The transfer of plants from (–N) to (+N) medium quickly restored leaf proline concentration, with a transient overshoot exceeding the proline level in control plants (Fig. 2). δ-OAT activity was poorly variable in control (Fig. 3). During the five last weeks of treatment, plants (–N) showed a high δ-OAT activity relatively to

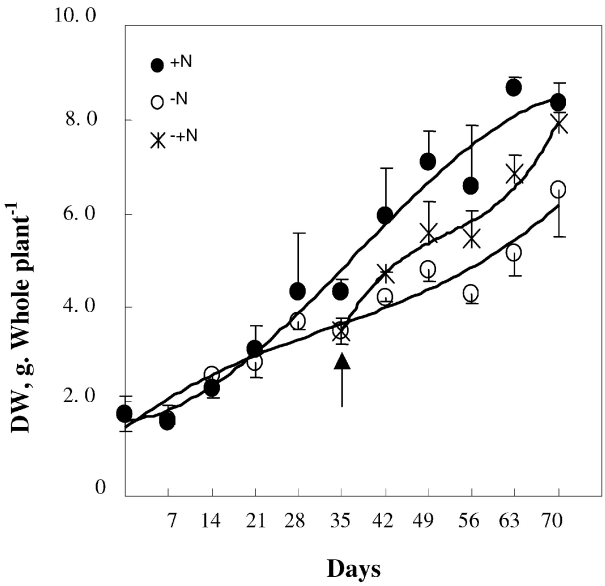

Figure 1. Nitrogen availability effect on whole plant dry matter production (Mean ± S.E., $n = 5$). Plants were grown on nutrient solution added with 400 mM NaCl. The arrow indicates the transfer of the plants previously subjected to nitrogen deficiency (0.28 mM) to appropriate N nutrition (14.4 mM).

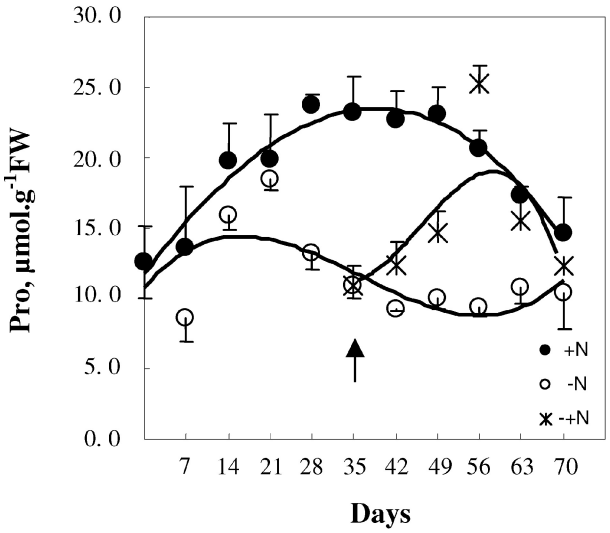

Figure 2. Nitrogen availability effect on proline concentration in leaves (Mean $\pm$ S.E., $n = 3$). Plants were grown on nutrient solution added with 400 mM NaCl. The arrow indicates the transfer of the plants previously subjected to nitrogen deficiency (0.28 mM) to appropriate N nutrition (14.4 mM).

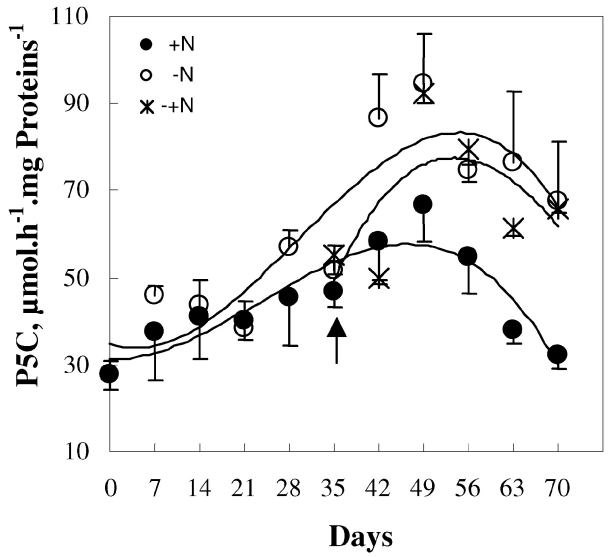

Figure 3. Changes in δ-OAT activity (µmol Pyrroline-5-Carboxylate (P5C) h^{-1} mg^{-1} protein) with nitrogen availability. Plants were grown on nutrient solution added with 400 mM NaCl. The arrow indicates the transfer of the plants previously subjected to nitrogen deficiency (0.28 mM) to appropriate N nutrition (14.4 mM).

that of control plants. The highest values of δ-OAT activity were concomitant with the lowest contents of proline. In plants supplied with N, an increase δ-OAT activity was associated with high proline concentrations. The ProDH activity presented a complex, peaking two fold in the first 3 weeks, then after 2 months of culture.

Water stress

The depressive effect of water deficit on the whole plant biomass appeared after 8 days of treatment (Fig. 4). After 40 days of water stress, the dry matter production of dehydrated plants was 44 % of the control. Rehydrating the plants after 28 day-long water stress allowed growth recovery, although the dry weight of the rewatered plants did not reach the level of the control. In control plants, proline accumulation remained almost unchanged during the whole period of treatment (about 10 μ mol. g^{-1} FW) (Fig. 5). Water deficit induced an increase in proline levels which grew with time. At the end of the experiment, plants submitted to water deficit accumulated twice more proline than control. Proline concentration in rehydrated plants decreased quickly, to values close the control. Contrasting behavior was revealed for ProDH protein, which was high in control, low in stressed plants, and high in rewatered plants.

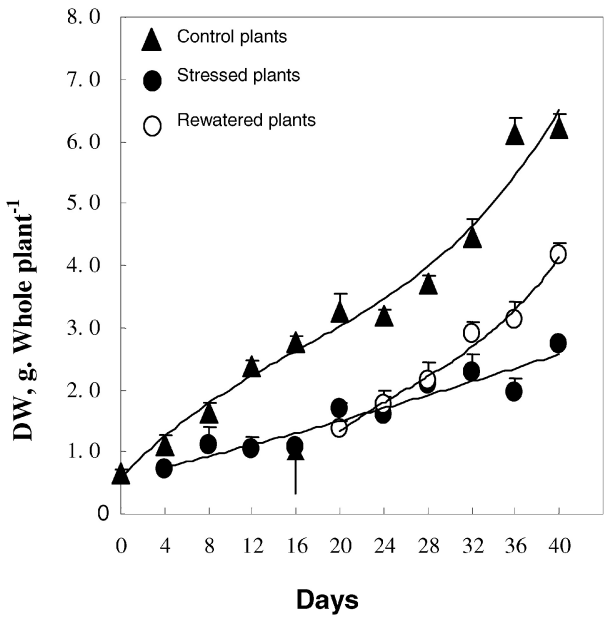

Figure 4. Water deficit stress effect on whole plant dry matter production (Mean ± S.E., $n = 3$). Controlled plants: plants grown on 100 % field capacity (FC), Stressed plants: plants grown on 25 % FC, rewatered plants: plants cultivated during 16 days on 25 % FC, and then transferred on 100 % FC. The arrow indicates the rewatering of the plants previously subjected to water deficit.

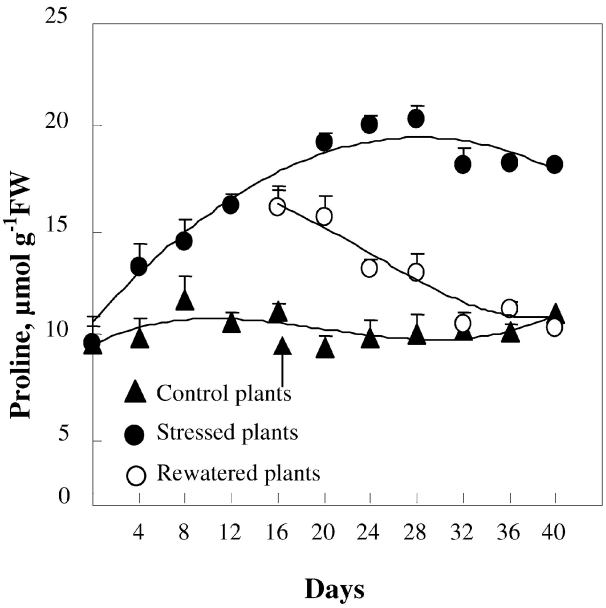

Figure 5. Changes in of *S. portulacastrum* leaf proline content (µmol g^{-1} FW) with water availability in the culture substrate (Mean $\pm$ S.E., $n = 5$). The arrow indicates the rewatering of the plants previously subjected to water deficit.

Discussion

Our results show that plants subjected to 400 mM NaCl (plants +N) expressed the same potentialities of growth as those cultivated in absence of salt. So, salt constraint did not affect the production of biomass at *S. portulacastrum*. It involved a significant increase in the contents of proline at the plants. But this phenomenon depends on the availability of the nitrogen. Indeed, at the plants submitted to a nitrogen deficiency as well as the growth, the accumulation of the proline was limited compared to (+N) plants. The water deficit reduced considerably growth without leading to visual toxicity symptoms (chlorosis or necrosis). It induced also a significant increase in proline concentration which reached 20–25 µmol.g $^{-1}$ FW. These results indicate that the plant reacts to the salt and water constraints by an accumulation of proline, and they suggest that the availability of nitrogen was essential to this response. To evaluate whether the accumulation of proline is an active process, we measured the activity of δ-OAT and ProDH, enzymes involved respectively in proline biosynthesis and catabolism. The δ-OAT activity increased under stress. However, in N deficient plants we showed also an increase in δ-OAT activity concomitant with a decrease in proline concentrations. These data suggest that this enzyme, normally involved in proline biosynthesis, can be also implied in its catabolism. This behavior was observed only in the animal cells. Indeed, δ-OAT interconverts P5C into ornithine and, therefore

plays an important role in both synthesis and degradation of proline [25]. During water stress, an inhibition of ProDH activity was concomitant with the absence of the protein band of ProDH. The increase of ProDH activity and the higher intensity of the protein band of this enzyme observed in plants subjected simultaneously to salt stress and N deficiency suggest that the nitrogen supply eliminate the inhibitory effect of salt on this enzyme. ProDH is normally induced by proline. However, Peng et al. [26] showed that this induction doses not occur under osmotic stress. This is in agreement with our result obtained in *S. portulacastrum* submitted to water stress. But under salt stress associated to N deficiency, an over-expression of ProDH was showed. According to Ahmed and Hellebust [27], 90 % of carbon and nitrogen in the soluble compounds are represented by amino acids. The degradation of the proline in glutamate is, thus, a potential source of energy.

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Biosaline Agriculture and Salinity Tolerance in Plants
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Linkage studies of structure, isoenzymatic diversity and some biotechnological procedures for *Salsola* species under desert saline environments

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Introduction

One of the main undesirable consequences of the process of desertification in the Central Asia countries is an amplification of salinity process resulting in a wide development of saline soils. The amplification of the salinity process under conditions of aridization of climate is caused by the high maintenance of salts both in the surface and in subsoil waters of dry lands of the Aral Sea Basin [1]. Besides that, the recent overuse of Amudarya, Zerafshan and Syrdarya river water has resulted in the waterlogging and secondary salinization (human caused) salt/affected lands on whole adjacent territories. Effects of these impacts include alteration or destruction of vegetation, frequent disappearance of many useful, endemic and relict species of desert plants, and consequently, establishment of annual plant communities dominated by weeds and exotic species. Up to 15,000 ha of pastures are annually affected by salinity and waterlogging that resulted in the reduction of population of more than 1,500 species of mammals, birds and plants in the whole of the Central Asia region [2, 3].

Today, large areas of the southern sandy Kyzylkum desert ecosystems have been negatively affected by intensive urbanization, overgrazing by livestock, cutting shrubs on fuel, cotton monoagriculture, soils and air pollution. Such type of desert human transformed ecosystems is frequently dominated by leaf-succulent plants like species of genera *Salsola*, *Climacoptera*, *Gamanthus*, *Halocharis* and many other wild native halophytes. It was suggested that harsh desert environmental factors, especially high salinity of soils, limit plant growth and the production of viable seeds. The lowest sexual reproduction ability, seed germination and, consequently, insignificant seedling survival under natural desert condition were noted for Asiatic haloxerophytes species [4–7].

We suggest that the initial exploration of natural plant cellular mechanisms affecting the phytoremediation of elemental and/or organic pollutants is quite promising for the use of desert plants in large-scale environmental clean-up efforts. Native plants have the advantage of being highly adaptive to the local climatic and edaphically contaminated and salt/affected environments.

Our previous data on the effect of desert stress factors on microscopic and submicroscopic structure of floral organs show that dryland plants and species of genera *Salsola* in particular, provide an excellent model for analysis of natural salt stress impact on the plant cellular characteristics [8–11]. However, clearly distinguishing multitudes of populations and evident polymorphism of all organs of arid species still remains very difficult to be managed, as adequate traits are lacking. High anatomical and morphological variability for many halophytes are often taken as plant adaptation to harsh desert environments that increase their chances to endure the stress imposed by salinity [7, 10, 12]. However, it is not clear to what extent this variability represents genetic, developmental or influence of environmental variation. Furthermore, little is known about variability of embryological features and the specificity of their reproductive strategies in response to arid salt stress factors. Also, there is not enough experimental data describing the procedures of plant micropropagation and callus induction for desert plants, in particular for representatives of Chenopodiaceae. Some research on *vitro* tissue culture technology was conducted on species of genera *Atriplex*, *Kochia* and *Suaeda* [13, 14]. The biotechnological procedures for *Salsola* arid species are still unknown. The main objectives of the present paper are to examine some natural mechanisms of plant salt tolerance on morphological and genetic levels, biotechnological experiments coming to the point of practical use and theoretical knowledge concerning plant resistance and tolerance to salt stress.

It was expected that the study of reproductive strategies of plants and selection of superior genotypes from natural deserts populations coupled with clonal propagation through tissue culture may offer an alternative way for improvement and conservation of arid salt/affected lands in Uzbekistan.

Materials and methods

Various populations of annual chenopods, *Salsola lanata Pall*. (*Climacoptera lanata*), *S. praecox Litv*. and *S. pestifer*, *A. Nelson* (*S. iberica* (*Sennen et Pau*) *Botsch*.), growing under foothills semidesert and sandy desert environments (South-Eastern Kyzylkum) were analyzed. The flower organs of four woody-shrubs species of genus *Salsola* were vacuum-coated with gold and analyzed by JEOL JSM-T330 scanning electron microscope (SEM).

Variability of eight enzymatic systems, 6PGD (E.C. 1.1.1.44), MDH (E.C. 1.1.1.37), GDH (E.C. 1.4.1.2), G6PD (E.C. 1.1.1.49), PGM (E.C. 5.4.2.2), PGI (E.C. 5.3.1.9), GOT (E.C. 2.6.1.1), DIA (E.C. 1.6.99) on the basis of starch gel electrophoresis of isoenzymes from randomly chosen embryos (30–35 for each population) was studied. Enzymes were extracted by homogenization of single embryos in 80 ml of extraction buffer EDTA, KCl, $MgCl_2$, TRITON, PVP, TRIS-HCl. Isoenzymes were separated in 10 % starch gel using two buffer systems. Staining

of particular enzymes and genetic interpretation of the results followed the standard techniques [15–17].

In our scientific work we used technology of plant micropropagation by seeds. The surface-sterilized embryos were placed in Petri dishes (6 cm diameter) on two types of solid medium. One of them consisted of only agar (8 g/l), another medium contained macro and microelements and vitamins according to Murashige and Skoog – MS medium; sucrose (20 g/l), agar (8 g/l) and kinetin (0.5 mg/l). Their pH was adjusted to 5.5 before autoclaving. Embryos were grown in dark condition at $24 \pm 1^{\circ}$ C. The seedlings were transferred on two types of micropropagation MS-medium, one of which consisted of IAA (0.05 mg/l) and BAP (0.1 mg/l), but another one – IAA (0.05 mg/l) and 2iP (0.01 mg/l). The culture was maintained in the air-conditioned culture room at $24 \pm 1^{\circ}$ C, 70 % relative humidity, with 16 h light/8 h dark photoperiod and 30 μmol$\cdot$m$^{-2}\cdot$s^{-1} intensity. In order to induce callus, the leaf and shoot explants were placed onto various callus induction MS-media consisting of different concentrations of plant growth regulators: BAP (2.0 and 5.0 mg/l), kinetin (2.0 mg/l), 2iP (1.0 mg/l), IBA (1.0 mg/l) and 2,4-D (2.0 mg/l) in dark conditions at $25 \pm 1^{\circ}$ C for 6 weeks. Then these callus cultures were transferred onto MS-medium supplemented with 2.0 mg/l 2,4-D to get a stable viable callus culture. In order to obtain plant regeneration, callus culture were transferred onto MS-medium consisted of cytokinin BAP (2.0 mg/l) and placed at $24 \pm 1^{\circ}$ C with 16 h light/8 h dark photoperiod. All analyses have been conducted at the Department of Botany of Adam Mickiewicz University in Poznan, Poland.

Results and discussion

The species studied by us are widespread haloxerophytes growing in open communities of inland saltmarshes and/or marshy-steppe communities of Aral-Caspian regions. All of them are considered useful for rehabilitation of arid/semiarid degraded and salt/affected lands. They possess superficial root and can be established from direct seeding and also are capable of self-sowing. All examined taxa had a low rate of field seed germination and were more tolerant to higher soils salinity at early seedling and growing stages [8, 18]. Seed reproduction can be by self-pollinating and out-crossing. *S. lanata*, forming a distinct and almost monospecific dominant plant community on desert salt/affected lands, showed a highly competitive vigor to such environments. *S. pestifer* and *S. praecox* with slender leaves, occur on dry, sandy, mostly in moderate saline soils. *S. praecox* mostly grows on sandy solonchaks of Iran-Turanian and Transcaspian lowlands. Its native wild spread populations are often small and isolated. Furthermore *S. pestifer* and *S. praecox* taxonomically are so closely related that sometimes it's difficult to distinguish their ecoforms. Both species possess both high vegetative and reproductive organ polymorphism.

Genetic and cytological diversity

Our experimental data shows that the examined enzyme systems, particularly 6PGD, DIA, G6PD, GDH, GOT, MDH, PGM, and PGI, were coded by different number of

loci: 14 in *S. lanata*, 17 in *S. pestifer* and 16 in *S. praecox*. The enzyme PGI is coded by three loci only in *S. pestifer* populations and two loci of G-6PD was founded in *S. lanata* populations. Moreover, in *S. lanata*, the enzymes GDH, GOT, PGI and PGM are coded by different numbers of loci than for *S. pestifer* and *S.praecox*. The general pattern of isoenzymatic bands of *S. pestifer* and *S. praecox* were similar for all loci, except PGI A, which was not detected for *S. praecox*. The proportion of polymorphic loci (0.95 criterions) was rather low: 5.9 % for *S. pestifer*, 7.1 % for *S. lanata* and 12.5 % for *S. praecox*. The mean number of alleles and genotypes per locus was not high either: from 1.1 in *S. pestifer* and *S. praecox* to 1.2 in *S. lanata* for both parameters. Generally, we found 18 alleles and 18 genotypes for *S. pestifer* and *S. praecox*, and 17 alleles and 7 genotypes for *S. lanata* in all loci of the examined enzyme systems. *S. lanata* and *S. pestifer* have similar values of genotype polymorphism index, Pg (0.025 and 0.029, respectively), as opposed to *S. praecox*, which has the highest genotype polymorphism (0.054). Values of genetic distance based on allele DN and genotype DH frequencies are summarized in Table 1. In both cases the smaller genetic distance was found for the pair *S. lanata – S. pestifer* and the largest for *S. pestifer – S. praecox.*

Table 1. Variability of genetic parameters of Asiatic *Salsola* species

Populations	% of loci polymorphic*	A/L	G/L	H_e	H_o	F	P_g
S. lanata	7.1	1.2	1.2	0.019	0.031	−0.675	0.025
S. pestifer	5.9	1.1	1.1	0.023	0.129	−4.733	0.029
S. praecox	12.5	1.1	1.1	0.033	0.159	−3.768	0.054

Table 1 gives the main genetic parameters calculated for the studied species: A/L-mean number of alleles per locus, G/L-mean number of genotypes per locus, He-expected heterozygosity, Ho-observed heterozygosity, F-Wright's fixation index, Pg-genotype polymorphism index.

Genetic similarities between examined species are surprisingly high: 0.996–0.999 according to Nei (IN) and 0.988–0.999 according to Hendrick (IH) [19], especially in comparison to values given by Crawford [20] for *Chenopodium* spp (0.35–0.97). Genetic similarities calculated for *S. lanata*, *S. pestifer* and *S. praecox* are as high as for conspecific populations of several *Chenopodium* species [20]. Genetic distances between the studied taxa are much lower (0.0007–0.0029) than the values estimated by Nei [21] between species (0.1–0.2) and between subspecies (0.02–0.1). Such a high isozyme monomorphism of *Salsola* species may be due to very narrow saline environmental conditions, under which only highly specialized organisms can survive [22]. This conclusion is in concordance with the hypothesis of Hamrick [19]. The authors suggest that strong directional selection might result in lower levels of genetic variation in arid plant populations. According to Golding [23] environment factors also shape frequencies of alleles, thus taxa experiencing similar conditions may exhibit similar allele frequencies. The above genetic data suggest that a significant component of the great morphological variation of the examined taxa may result from phenotypic plasticity. By contrast, Rilke and Reimann [12] present

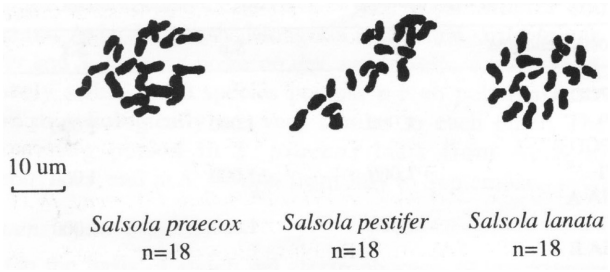

Figure 1. Karyotype characteristics for some Asiatic annual species of genus *Salsola*

that differential saline tolerance of *Salsola kali* from northern Europe suggests some genetic differentiation.

Thus, our results show rather low levels of isoenzymatic and genetic variation of investigated populations of annual *Salsola* species. Nevertheless, this study demonstrates the usefulness of electrophoretic technique in exploring genetic diversity and taxonomic position of many close related species of the Chenopodiaceae family. Obviously, our findings require further studies on wider plant material in respect to genetic variation and its relation to environmental conditions.

The preliminarily karyological analysis revealed that all three examined species have the same number of chromosomes: 2n = 18 (Fig. 1). However, further investigations are necessary.

Variability of reproductive organs and its adaptive trends

By means of scanning electron microscopy (SEM) analysis it was defined that fruit tepals morphology and anatomy plays a significant role in the species delimitation. Besides this, many fruits' morphological and structural traits are indicated to the adaptive level of *Salsola* species to deserts saline environments that in some way determine their seed viability and germination ability. The studied annual C4-*Salsola* species showed an evident polymorphism in the morphology, density and sizes of papillae, hairs, trichomes and salt glands (shape of their head: mainly clavate or capitate). Abundant papillae prickle hairs and secreted salts on the ridges of adaxial bracts/bracteoles are described (Fig. 2). Frequently, salt glands are globose or club-shaped and readily distinguishable from unicellular papillae and sharp-pointed prickles. This is a prominent mechanism of salts accumulation both on the surface and/or internal cellular tissue (epidermis and parenchyma) in the form of crystals (usually containing oxalates), which are often secreted chiefly in the form of clusters both in solution, sands or others types are marked. The accumulation of toxic salts into the reproductive organs, embryo tissues in particular, has not been detected. It was also found that the species of *Climacoptera* develops mostly unicellular non-glandular trichomes (Fig. 3) or prickle hairs possessing a smooth (warted) succulent surface, while the dry/sclerified species (*S. pestifer* and *S. praecox*) have an indulating epidermal surface with a tall adaxial ridges alternating with deep grooves. The salt glandular structures and salt secretion on the bract/bracteoles of these species

Figure 2. The morphology of vesicular and short peltate trichomes with sunken stomata on bracts in *S.pestifer* (Buchara ecotype)

Figure 3. SEM micrograph showing surface features and morphology of non-glandular, unicellular hair of bracts in *Climacoptera lanata* (Buchara ecotype)

become abundant when plants are exposed to high saline environments. We supposed that these parameters could be used as discriminating characters between different ecological halophytes *Salsola* groups. Variation in the indumentum density is believed to be mainly the effect of stress/desert environmental factors and/or even herbivores pressure [24].

A lyzicarpous utricle, winged and monospermous fruit are characterized for all studied species. The seeds are small, dark-brown, orbicular and horizontally arranged. The embryo is large and spirally coiled. A strong exogenic type of dormancy was found for these annual Asiatic *Salsola* species. This type of seed dormancy is

closely related to the 'perianth segments' structure and its chemical composition presents some physical barriers and chemical inhibitors (phenolic compounds, abcisic and nicotinic acids, surplus of mineral salts), reducing the water and gaseous exchange to the embryo [25]. Seed loses their viability 6–8 months after harvesting. Long-term stratification, 1.0–2.5 months under 2–4° C/15–28° C night/day cycle and/or seed's wash (2–3 times/10 min) with distilled water from inhibitors positively contribute to the seed germination. The destruction of intactness of seed coat or removal of fruit cover is also effective to increase seed germination. Dry storage (under 20/28°C in special hermetic cameras) of seeds gives positive results to increase and/or keep its germinability both in laboratory and field conditions.

Plant micropropagation and callus induction procedures

We found that seeds of all examined species germinate within 1–2 days. The rate of seed germination was higher on pure agar than on MS medium with kinetin as a growth regulator. It was more peculiar for *S. pestifer* (sand desert population). Shoot proliferation from seeds was noticed on both used micropropagation mediums. As is known, different parts of a plant such as roots, annual and perennial shoots, leaves, flowers, and ovary have been used as explants to obtain callus culture [26–29]. In our experiments we have used fragments of shoots and leaves to induce callus. It was determined that the growth regulators in MS medium have a different influence on callus induction even within limits of the same species. As shown in Table 2, callus initiation of *S. pestifer* (foothills semidesert population) was much evident on the medium supplemented by BAP (2.0 mg/l); while IBA (1.0 mg/l) was the most suitable growth substance that induces callus culture of *S. pestifer* (sand desert population).

Process of callus induction depended also on explant types. It was determined that callus culture for both populations of *S. pestifer* showed the best development from shoot explants. At the same time the leaf explants of *S. lanata* induced callus culture more often when comparing with shoot explants. It is important to note that callus formation started within 1 week of inoculation on MS medium supplemented by BAP (2.0 mg/l) as a growth regulator for all of the studied plants. The earliest sign of callus formation from cuttings explants of all species has been visible within 1 week on MS medium supplemented by BAP (2.0 mg/l). This medium was also the most suitable medium for callus induction from both shoot and leaf explants for foothills semidesert populations of *S. pestifer*. MS medium with IBA, however, seemed to be one of the optimal medium for callus culture induction in the case of the sandy desert populations of *S. pestifer* (Tab. 2).

According to some authors, the auxine 2,4-D is most frequently used to initiate plant callus culture [28, 30]. For our purpose, 2,4-D appeared to be the most suitable growth regulator to induce callus culture of *S. lanata* and to obtain the stable viable callus for all three investigated species. The explants of *S. pestifer* produced two type of callus: a white, transparent, soft and white, compact. For *S. lanata*, a yellowish, compact callus culture was observed.

The plant regeneration both from lateral buds and callus culture of *S. lanata* was obtained by using MS medium on 2.0 mg/l BAP.

Table 2. Callus induction from shoot and leaf explants on different types of medium

MS medium with growth regulators (mg/l)	Shoot explants		Leaf explants	
	Total, number	With callus induction (%)	Total, number	With callus induction (%)
Salsola pestifer (foothills semidesert population)				
BAP (2.0)	46	100.0	77	81.8
BAP (5.0)	7	42.9	46	15.2
Kin (2.0)	20	40.0	20	65.0
2iP (1.0)	19	–	20	–
IBA (1.0)	20	95.0	20	35.0
2,4-D (2.0)	13	–	13	–
Salsola pestifer (sand desert population)				
BAP (2.0)	48	91.7	90	68.9
BAP (5.0)	2	–	49	–
Kin (2.0)	19	94.7	16	100.0
2iP (1.0)	18	100.0	14	28.6
IBA (1.0)	25	100.0	21	100.0
2,4-D (2.0)	18	94.4	20	100.0
Salsola lanata				
BAP (2.0)	58	5.2	84	38.1
BAP (5.0)	11	–	21	4.8
Kin (2.0)	18	–	17	64.7
2iP (1.0)	23	–	20	80.0
IBA (1.0)	19	31.6	19	31.6
2,4-D (2.0)	18	100.0	15	93.3

Conclusions

Studies on reproductive organs' structural analysis and genetic variation of three Asiatic haloxerophytes of the genus *Salsola* demonstrate the usefulness of SEM and electrophoretic techniques in exploring genetic diversity and taxonomic position of many close related species of the genus *Salsola* from the Chenopodiaceae family. Some structural traits of salt glands/hairs morphology could be used as discriminating characters between different ecological *Salsola* halophytes groups.

Preliminarily methodological work on plant micropropagation and callus induction showed the potential utilization of Asiatic annual haloxerophytes in selection of salt-tolerant genotypes for dealing with rehabilitation of saline affected lands in the continental desert climate of Uzbekistan.

Thus, our results should enable more rigorous selection of salt/drought tolerant plants for rehabilitation of saline/sodic sandy desert lands in Uzbekistan. The received data can be integrated in a global database to draw a full description of the genetic variation of desert plant resources on a Central Asian scale. It is an important step towards practical and accurate methods for the determination of the key problem in a modern rangelands management in Uzbekistan.

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Biosaline Agriculture and Salinity Tolerance in Plants
Edited by M. Öztürk, Y. Waisel, M.A. Khan and G. Görk

Kinetics of the antioxidant response to salinity in *Crithmum maritimum*

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Introduction

Salinity limits the production of approximately 40 % of the world's agricultural land [1]. In order to overcome the decline of cultivated areas and the high demands for food and energy, a particular interest was accorded to the salty lands. To re-green these areas, two strategies have been developed: i) the genetic manipulation of common crop species for increased salt tolerance, and ii) the utilization of naturally salt-tolerant species (halophytes) [2].

Mechanisms of salt tolerance are of two main types: those minimizing the entry of salt into the plant, and those preventing the salt concentration in the cytoplasm. Halophytes, naturally salt tolerant plants, express both these properties. They exclude salt well, but effectively compartmentalize in vacuoles the salt that inevitably gets in, which ultimately allows them to grow for a long period in saline soils.

The involvement of antioxidative response systems (ARS) in salt tolerance was often reported in halophytes [1, 3–5]. The primary components of this defence system include carotenoids, ascorbate, glutathione, and tocopherol as well as antioxidant enzymes such as superoxide disumutases (SOD), catalase, peroxidases and the enzymes of the ascorbate glutathione cycle (e.g., ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase) [6, 4]. Plants containing high concentrations of antioxidants show considerable resistance to the oxidative damage caused by the activated oxygen species [7, 5]. Recent reports on the response of plant antioxidant enzymes to salinity have indicated several distinct patterns, which varied according to the species and the tissue analyzed [8, 9]. The aim of this study was to investigate the changes in activities of the antioxidant enzymes, SOD, catalase (CAT, EC 1.11.1.6) and peroxidase (POD, EC 1.11.1.7) in the leaves and roots of *Crithmum maritimum*, a perennial wild halophyte, in response to increasing NaCl.

Material and methods

Seeds of *C. maritimum* collected from rocky coasts of Tabarka (160 km north of Tunisia) were sterilized in 0.2 % (w/v) sodium hypochlorite for 3 min and germinated on filter paper in Petri dishes in a growth chamber at controlled conditions (16 h/8 h light/dark regime; 200 μmol·m^{-2}·s^{-1} photosynthetic active radiation (PAR); 15–25° C temperature; 70–90 % relative humidity). Two-leaf seedlings were transferred to 5 l plastic pots (5 plants per pot) and were hydroponically cultivated, using aerated Hewitt nutrient solution (pH 7.3, EC 2.7 mS.cm^{-1}). Plants were separated in three groups irrigated with a nutrient solution supplemented with different concentrations of NaCl (0, 50 and 200 mM). To avoid osmotic shock, salt was increased gradually up to the final salt concentration. The nutrient solutions were renewed every 4 days. Intermediate harvests were carried out after 0, 10, 20, 30 and 60 days of salt treatment. Fresh samples (shoot and root) were immediately frozen in liquid nitrogen and stored at –80° C until biochemical determination.

The plant material was extracted at 4° C in 100 mM Tris-HCl buffer (pH 8.0) containing 10 mM EDTA, 50 mM KCl, 20 mM $MgCl_2$, 0.5 mM PMSF, 1 mM DTT, 0.1 % (v/v) Triton X-100, and 10 % (W/W) PVP. The ratio of plant material to buffer was 1:3. The homogenate was centrifuged at $14,000 \times g$ for 30 min at 4° C and the supernatant was used for enzymatic analysis. Three replicates per treatment were used. Protein concentration was determined using bovine serum albumin as a standard [10].

Catalase activity was performed by following the H_2O_2 disumutation at 240 nm in a reaction mixture (3 ml) composed of 50 mM sodium phosphate buffer (pH 7.0), 30 % H_2O_2 (DO 0.52–0.55 at 240 nm) [11]. CAT activity was expressed as units (μmol of H_2O_2 decomposed per min) per mg of protein. Peroxidase activity was determined spectrophotometrically by measuring the oxidation of o-dianisidine (3, 3'-dimethoxybenzidine) at 460 nm [12]. POD activity was expressed as units (μmol of oxidized dianisidine per min) per mg of protein.

Results

H_2O_2-detoxifying enzyme responses in roots

CAT activity increased 20 and 30 days after the start of NaCl treatment, respectively in controls and plants treated with 50 mM NaCl, and reached a maximal value at the end of treatment (60 days) (Fig. 1). POD activity increased gradually with time in both treated and control plants. The effect of salt stress on POD activities was only perceptible at the end of treatment (Fig. 2). On the other hand, the two enzyme (CAT and POD) activities showed variations in the 200 mM NaCl-treated plants, especially at the beginning of the treatment.

H_2O_2-detoxifying enzyme response in shoots

CAT activity of both control and NaCl-treated plants increased slightly 10 days after the onset of the treatment (Fig. 3). Thereafter, CAT activity remained constant until

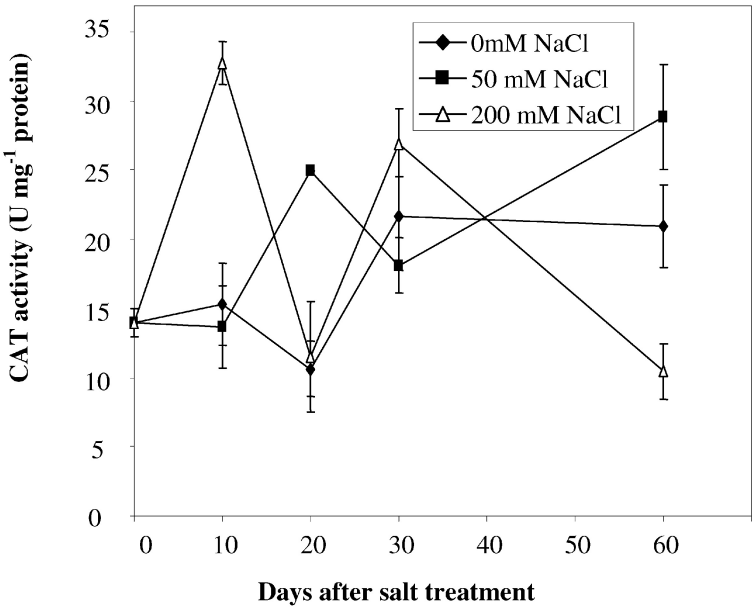

Figure 1. Changes in CAT activity (Unit mg^{-1} protein) of *C. maritimum* roots in response to NaCl. Means of 5 replicates ($\pm$ SE, P $=$ 0.05)

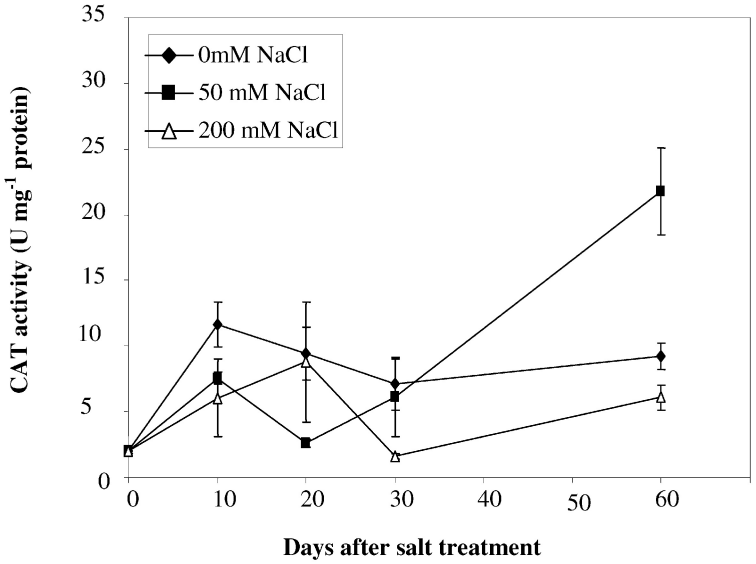

Figure 2. Changes in CAT activity (Unit mg^{-1} protein) of *C. maritimum* shoots in response to NaCl. Means of 5 replicates ($\pm$ SE, P $=$ 0.05)

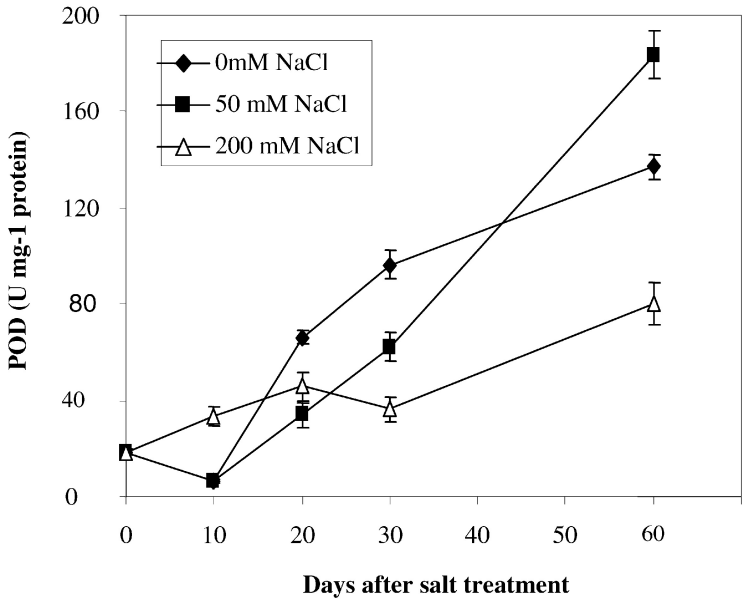

Figure 3. Changes in POD activity (Unit mg^{-1} protein) of *C. maritimum* roots in response to NaCl. Means of 5 replicates ($\pm$ SE, $P = 0.05$)

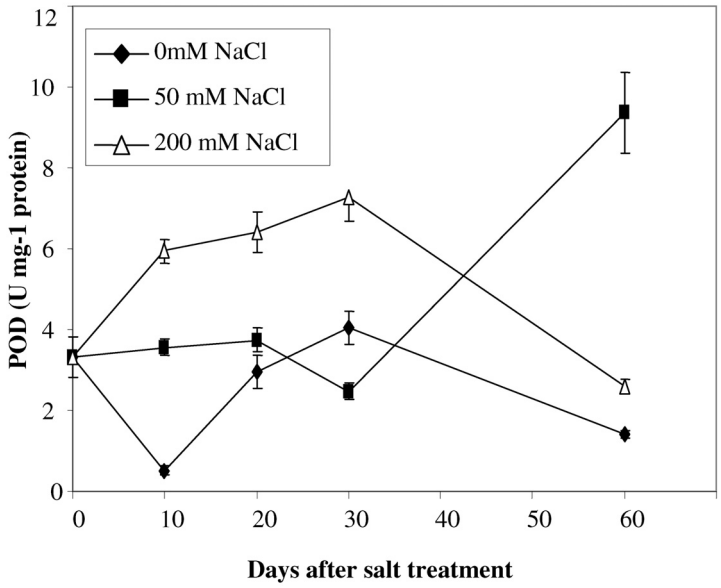

Figure 4. Changes in POD activity (Unit mg^{-1} protein) of *C. maritimum* shoots in response to NaCl. Means of 5 replicates ($\pm$ SE, $P = 0.05$)

the end of the treatment in both control and 200 mM NaCl-treated plants. In addition, CAT activity increased substantially the plants treated with 50 mM NaCl (Fig. 3). Changes in POD activities were time and dose-dependant. At the beginning of the treatment, the highest activity was found in plants treated with 200 mM. The most important activities at the end of salt treatment were recorded in shoot extracts from plants treated with 50 mM NaCl (Fig. 4).

Discussion

Several mechanisms are developed by plants to detoxify the reactive oxygen species (ROS). It is clearly important to establish whether the exposure of plants to salinity causes a detrimental or stiumulatory effect on the enzymes involved in this detoxification process. Salt stress tolerance has been correlated to an improved oxidative stress response in several crops [1, 13–15, 5]. SOD, CAT, and POD are among the major antioxidant enzymes involved in scavenging ROS [16]. Previous studies investigating the impact of NaCl on CAT activity in higher plants, reported a decrease in activity, but with a variation during the time of treatment. The decline in CAT activity due to salt stress in cotton, in sunflower, and *Bruguiera parviflora* [17] agrees with our results in *C. maritimum*, when treated with 200 mM NaCl. Furthermore, similarly to *Sedum album* [18], an inductive response in CAT at low NaCl concentration (50 mM) was found in *C. maritimum.*

Unspecific peroxidase activity has been used as a general indicator of stress induced by high temperature, salinity and drought [19, 20]. A salt-induced increase of peroxidase activity was reported to occur in foxtail millet [21] and rice [22]. Similarly, our data show a high level of induction in POX activity in *C. maritimum*. The extents of increase in activity appear to be significant in relation to the duration of the treatment and the concentration of salt.

Our findings strongly support an induced increase in H_2O_2-scavenging enzyme activity (CAT and POD) in both leaves and roots of *C. maritimum* cultivated in the presence of 50 mM NaCl. Currently, it is not known whether the increase might be due to an upregulation of the genes controlling the synthesis of these enzymes or to an increased activation of constitutive enzyme pools. Since other studies have indicated that salt stress increases the synthesis of certain proteins in some plants [23], it is likely that the salt-induced increase in the antioxidant enzyme activities observed in *C. maritimum* might be due to changes in the antioxidant gene encoding the enzyme activities.

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Biosaline Agriculture and Salinity Tolerance in Plants
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An overview of the coastal zone plant diversity and management strategies in the mediterranean region of Turkey

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Introduction

The Mediterranean basin has served as the cradle for well known civilizations [1]. Its favourable environmental conditions have attracted humans towards the coastal parts for thousands of years and they exploited the land very severely, thus resulting in the degradation of this complex ecosystem [2]. During the last few decades the basin has become a focus of attention for scientists and several projects have been followed [3–6]. Turkey is one of the countries with a long coastline in the basin including the Mediterranean Sea and Aegean Sea with a total length of 5,191 km [4]. The high mountain ranges run in close proximity to the shoreline allowing some of the rivers to form fertile alluvial plains. Due to the varying geological features, the coastline is highly indented embodying several bays and coves serving as the main area for tourism and recreation as well as other coastal uses.

The area is endowed with a rich plant diversity. Out of these, the saline and dune vegetation occupy an important place due to their natural recreational values as well as importance in the production of food, fodder, fibre and many other products [7].

Much work has been done on the flora of Turkey [8], but very few papers have been published on the coastal zone plant cover [9, 11–13]. In this paper, an attempt is made to present an overview of the coastal zone plant cover of the saline and dune habitats in the Mediterranean phytogeographical region of Turkey and suggestions for the management of this fragile ecosystem.

Study area

The study area covers the states of İzmir, Aydın, Denizli, Muğla, Antalya, İçel, Adana and Antakya located in the Mediterranean phytogeographgical region of Turkey extending from Canakkale up to the Syrian border and covering an area of nearly 500,000 km^2 with several varying biotopes [14].The area in general experiences a

typical Mediterranean climate with dry-hot summers and mild-rainy winters (Fig. 1). The precipitation regimes of Izmir, Aydın, Denizli, Muğla, Antalya and İçel follow the course as WSASu (W: Winter; S: Spring; A: Autumn; Su: Summer). In Adana and Antakya it is observed as WASSu (Tab. 1) [15, 16].

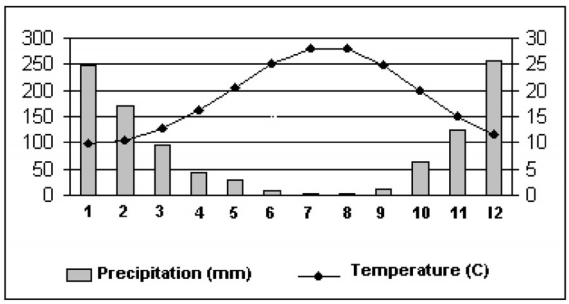

Figure 1. The temperature and precipitation values in the Mediterranean climate

Table 1. Precipitation regime of the study area (seasonal rainfall, mm) (1994–2000). From [16]

States	Winter (W)	Spring (S)	Summer (Su)	Autumn (A)	Precipitation regimes
İzmir	399.8	146.8	14.1	137.5	WSASu
Aydın	355.0	149.4	21.1	139.9	WSASu
Denizli	239.5	160.4	37.8	97.5	WSASu
Muğla	722.0	231.3	36.0	217.0	WSASu
Antalya	695.5	214.4	12.2	166.9	WSASu
İçel	356.7	127.5	25.8	114.4	WSASu
Adana	333.9	124.3	29.5	168.5	WASSu
Antakya	568.1	213.7	57.9	313.7	WASSu

Plant diversity

The studies undertaken by several authors have revealed that different ecological habitats along the Mediterranean coastal zone embody an exceptionally high biodiversity [4, 9–13]. The pristine coastal dunes and beaches are of great importance as the breeding grounds of the endangered marine turtles *Caretta caretta* and *Chelonia mydas*. The area at the same time shows a high endemism in plants [4]. The plant taxa collected from the coastal zone of the Mediterranean area were identified by using the *Flora of Turkey and East Aegean Islands* [8] as well as other relevant books on the flora of Turkey. The records from other published papers were also noted [10–13].

Table 2. List of plant taxa from the Turkish Mediterranean coast

Families	Taxa	Life form	Chorotype	Ecotype	Altitude (m above sl.)	Flowering time (months)
Amaryllidaceae	*Pancratium maritimum*	C	M	PM	0–5	6–10
Apiaceae	*Anethum graveolens*	T	IM	R	–	6–7
Apiaceae	*Bupleurum semicompotisum*	T	IM	H	200	3–6
Apiaceae	*B. euboeum*	T	M	H	0–1500	6–8
Apiaceae	**B. zoharii*	T	M	R	0–1200	6–8
Apiaceae	*Crithmum maritimum*	CH	IM	X		7–10
Apiaceae	*Daucus broteri*	T	M	X	0–100	6–8
Apiaceae	*D. carota*	T	IM	X	0–2000	6–9
Apiaceae	*D. littoralis*	T	M	X	sea level	
Apiaceae	*Eryngium campestre* var. *virens*	H	IM	X	0–1800	7–9
Apiaceae	*E. maritimum*	H	IM	PM	sea level	6–8
Apiaceae	*Falcaria falcaroides*	H	IM	H	380–1250	6–8
Apiaceae	*Pseudorlaya pumila*	T	M	X	sea level	3–6
Asclepiadaceae	*Cionura erecta*	CH	IM	H	0–1400	4–9
Asclepiadaceae	*Cynanchum acutum*	C	IM	H	0–1500	6–9
Asteraceae	*Ambrosia maritima*	C	M	PM	0–500	8–12
Asteraceae	**Anthemis ammophila*	T	M	PM	5	4–5
Asteraceae	*Aster tripolium*	H	ES	PM	–	6–9
Asteraceae	*Atractylis carduus*	CH	IM	PM	sea level	5
Asteraceae	*Bellis annua*	T	M	H	0–300	2–5
Asteraceae	*B. perennis*	H	ES	H	0–2000	3–8
Asteraceae	*Carlina lanata*	T	M	X	10–800	6–8
Asteraceae	*Centaurea aegialophila*	H	M	X	0–100	4–6
Asteraceae	*C. calcitrapa* subsp. *calcitrapa*	H	M	R	0–400	6–10
Asteraceae	*C. spinosa* var. *spinosa*	CH	IM	PM	sea level	6–7
Asteraceae	*Chondrilla juncea* var. *juncea*	H	IM	X	150–270	7–9
Asteraceae	*Chrysanthemum coronarium*	T	M	R	0–500	4–6
Asteraceae	*Crepis foetida* subsp. *commutata*	T	IM	X	0–1200	4–6
Asteraceae	*C. foetida* subsp. *rhoeadifolia*	T	IM	PM	0–2000	5–10
Asteraceae	*C. sancta*	T	IM	H	–	–
Asteraceae	*Hedypnois cretica*	T	M	PM	0–900	3–5
Asteraceae	*Inula crithmoides*	CH	IM	H	sea level	9–11
Asteraceae	*I. graveolens*	T	M	PM	0–800	8–10
Asteraceae	*I. viscosa*	CH	M	X	0–800	6–11
Asteraceae	*Otanthus maritimus*	CH	M	PM	sea level	5–11
Asteraceae	*Xanthium strumarium* subsp. *cavanillesii*	T	IM	R	0–950	7–10
Boraginaceae	**Alkanna pinardii*	CH	M	X	0–320	2–5

Table 2. Continued

Families	Taxa	Life form	Chorotype	Ecotype	Altitude (m above sl.)	Flowering time (months)
Boraginaceae	*A. tinctoria*	CH	M	X	0–800	4–7
Boraginaceae	*Anchusa aggregata*	T	M	PM	0–5	3–5
Boraginaceae	*A. undulata* subsp. *hybrida*	C	M	PM	0–900	5–7
Boraginaceae	*Echium angustifolium*	CH	IM	PM	0–870	8–12
Boraginaceae	*Heliotropium europaeum*	T	M	R	0–1400	6–9
Brassicaceae	*Cakile maritima*	T	IM	PM	sea level	6–8
Brassicaceae	*Maresia nana*	T	IM	PM	sea level	4
Brassicaceae	*Raphanus raphanistrum*	T	IM	X	0–400	3–5
Brassicaceae	*Sinapis alba*	T	IM	R	0–1400	2–4
Brassicaceae	*S. arvensis*	T	IM	R	0–1800	4–6
Caryophyllaceae	**Arenaria pamphylica* var. *turcica*	T	IM	X	0–800	4–8
Caryophyllaceae	**Dianthus crinitus* var. *pamphylica*	T	IM	X	0–215	3–5
Caryophyllaceae	*Silene kotschyi* var. *maritima*	T	IM	PM	sea level	5–7
Caryophyllaceae	**S. pompeiopolitana*	T	M	PM	sea level	4
Caryophyllaceae	*Spergularia marina*	H	CSM	H	sea level	3–6
Caryophyllaceae	*S. media*	H	CSM	H	sea level	5–8
Caryophyllaceae	*S. rubra*	T	IM	H	0–2500	4–8
Chenopodiaceae	*Artrocnemum fruticosum*	CH	IM	H	0–1100	8
Chenopodiaceae	*A.glaucum*	CH	IM	H		10
Chenopodiaceae	*Atriplex hastata*	T	IM	H	0–50	5–8
Chenopodiaceae	*A. lasiantha*	T	IM	R	0–1800	5–7
Chenopodiaceae	*Chenopodium album* subsp. *album*	T	IM	R	0–2000	5–8
Chenopodiaceae	*C. botrys*	T	IM	PM	0–1900	5–7
Chenopodiaceae	*C. murale*	T	IM	R	0–400	5–7
Chenopodiaceae	*C. opulifolium*	T	IM	R	sea level	5–8
Chenopodiaceae	*Halimione portulacoides*	CH	IM	H	0–900	6–8
Chenopodiaceae	*Halopeplis amplexicaulis*	T	IM	H	sea level	6–8
Chenopodiaceae	*Halocnemum strobilaceum*	CH	IM	H	0–1100	7–9
Chenopodiaceae	**Kalidiopsis wagenitzii*	CH	IM	H		5–6
Chenopodiaceae	*Kochia prostrata*	CH	IM	X	0–1900	6–8
Chenopodiaceae	*Petrosimonia brachiata*	CH	IM	H	0–900	6–9
Chenopodiaceae	*Salicornia europaea*	T	IM	H	–	7–9
Chenopodiaceae	*Salsola kali*	T	IM	R	0–1010	5–7
Chenopodiaceae	*S. soda*	T	IM	H	sea level	5–7
Chenopodiaceae	*S. ruthenica*	CH	IM	PM	0–1750	5–7
Chenopodiaceae	*Suaeda prostrata* subsp. *prostrata*	T	IM	H	sea level	6–9
Cistaceae	*Fumana thymifolia* var. *thymifolia*	CH	IM	PM	0–250	3–4

Table 2. Continued

Families	Taxa	Life form	Chorotype	Ecotype	Altitude (m above sl.)	Flowering time (months)
Cistaceae	*Helianthemum stipulatum*	CH	M	PM		4
Convolvulaceae	*Calystegia soldanella*	C	IM	PM	sea level	5–7
Convolvulaceae	*Convolvulus lanatus*	CH	Ss	PM	sea level	5
Convolvulaceae	*Cressa cretica*	T	IM	H	sea level	6–8
Convolvulaceae	*Ipomoea stolonifera*	T	M	PM	sea level	6–9
Cyperaceae	*Bolboschoenus maritimus* var. *maritimus*	C	CSM	H	0–2000	
Cyperaceae	*Carex divisa*	C	ES	H	0–2800	–
Cyperaceae	*Cyperus capitatus*	C	IM	H	6–8	1–5
Cyperaceae	*C. longus*	C	CSM	H	0–1850	5–9
Cyperaceae	*Eleocharis mitracarpa*	C	CSM	H	0–2400	4–9
Cyperaceae	*E. palustris*	C	CSM	H	0–2400	–
Cyperaceae	*Schoenoplectus litoralis*	C	IM	H	0–1370	4–10
Cyperaceae	*Schoenus nigricans*	H	IM	H	0–2000	3–7
Euphorbiaceae	*Euphorbia paralias*	H	M	PM	0–10	4–9
Euphorbiaceae	*E. peplis*	T	M	PM	sea level	6–9
Fabaceae	*Alhagi mannifera*	CH	IM	X	0–2330	6–8
Fabaceae	*A. pseudalhagi*	CH	IT	H	0–1200	6–8
Fabaceae	*Argyrolobium uniflorum*	H	Ss	PM	sea level	5
Fabaceae	*Astragalus epiglottis*	T	M	X		5
Fabaceae	**A. suberosus* subsp. *mersinensis*	T	M	X	0–1100	4–6
Fabaceae	**A. suberosus* subsp. *suberosus*	T	M	X	0–1500	4–6
Fabaceae	*A. subuliferus*	CH	M	X		4
Fabaceae	*Factorovskya aschersoniana*	T	M	X	sea level	1–2
Fabaceae	*Glycyrrhiza glabra* var. *glandulifera*	H	IM	PM	0–1800	6–7
Fabaceae	*Lotus corniculatus* var. *tenuifolius*	H	CSM	H	0–2750	4–9
Fabaceae	*Medicago littoralis* var. *littoralis*	T	IM	PM	sea level	4–6
Fabaceae	*M. marina* var. *marina*	H	IM	PM	sea level	2–6
Fabaceae	*M. minima* var. *minima*	T	IM	PM	0–1750	3–5
Fabaceae	*Melilotus indica*	T	IM	H	0–1750	2–5
Fabaceae	*M. messanensis*	T	M	PM	sea level	2–4
Fabaceae	*Ononis natrix* subsp. *hispanica*	CH	M	PM	sea level	5–8
Fabaceae	*O. variegata*	T	M	PM	sea level	4–6
Fabaceae	*Ornithopus compressus*	T	M	X	0–300	4–6
Fabaceae	*Prosopis farcta*	P	IM	H	0–1450	6
Fabaceae	*Trifolium campestre*	H	CSM	X	0–2200	2–4(–9)
Fabaceae	*T. resupinatum* var. *resupinatum*	T	IM	H	0–1500	5
Fabaceae	*Trigonella cylindracea*	T	M	PM	sea level	4–6

Table 2. Continued

Families	Taxa	Life form	Chorotype	Ecotype	Altitude (m above sl.)	Flowering time (months)
Fabaceae	*T. halophila*	T	M	PM	sea level	4–6
Frankeniaceae	*Frankenia pulverulenta*	T	IM	H	0–1000	7–8
Gentianaceae	*Blackstonia perfoliata* subsp. *perfoliata*	T	IM	H	0–900	4–10
Gentianaceae	*Centaurium spicatum*	T	IM	H	0–1070	7–8
Gentianaceae	*C. erythraea* subsp. *erythraea*	H	ES	H	0–900	5–8
Gentianaceae	*C. tenuiflorum* subsp. *tenuiflorum*	T	IM	H	0–1150	6–8
Geraniaceae	*Geranium dissectum*	T	IM	X	0–400	4–5
Geraniaceae	*Erodium cicutarium* subsp. *bipinnatum*	T	IM	PM	sea level	3–4
Guttiferae	*Hypericum polyphyllum ssp. polyphyllum*	CH	M	X	0–770	5–6
Guttiferae	*H. triquetrifolium*	CH	IM	X	0–1250	5–9
Illecebraceae	*Paronychia argentea* var. *argentea*	H	IM	PM	sea level	3–6
Juncaceae	*Juncus acutus*	C	IM	H	0–150	3–5
Juncaceae	*J. maritimus*	C	IM	H	0–1050	5–7
Juncaceae	*J. subnodulosus*	C	IM	H	0–100	6–7
Juncaceae	*J. subulatus*	C	M	H	sea level	4–6
Lamiaceae	*Lavandula stoechas* subsp. *stoechas*	CH	M	X	sea level	3–6
Lamiaceae	*Prasium majus*	CH	M	X	0–1300	3–7
Lamiaceae	*Salvia viridis*	T	M	X	0–1300	3–7
Lamiaceae	*Teucrium polium*	H	CSM	PM	0–2050	6–9
Lamiaceae	*T. scordium* subsp. *scordioides*	C	ES	PM	50–2350	5–9
Liliaceae	*Asparagus aqutifolius*	CH	R	X	0–1525	8–9
Liliaceae	*Urginea maritima*	C	M	PM	0–300	9–11
Orchidaceae	*Orchis palustris*	C	CSM	H	0–1950	6–7
Papaveraceae	*Papaver stylatum*	T	M	X	0–1200	3–6
Plantaginaceae	*Plantago coronopus* subsp. *commutata*	T	M	PM	0–800	2–7
Plantaginaceae	*P. coronopus* subsp. *coronopus*	T	IM	H	0–1750	4–11
Plantaginaceae	*P. crassifolia*	H	M	PM	0–900	5–10
Plantaginaceae	*P. cretica*	T	M	X	0–600	4–5
Plantaginaceae	*P. lagopus*	H	M	H	0–2000	4–8
Plantaginaceae	*P. lanceolata*	H	IM	H		
Plantaginaceae	*P. maritima*	H	IM	H	0–2400	5–8
Plantaginaceae	*P. scabra*	T	IM	X	0–1250	5–11
Plumbaginaceae	*Limonium angustifolium*	H	M	H	sea level	5–10
Plumbaginaceae	*L. bellidifolium*	H	ES	H	0–1010	6–9
Plumbaginaceae	*L. echioides*	T	M	H	0–200	4–7
Plumbaginaceae	**L. effesum*	H	M	H	0–750	7–8
Plumbaginaceae	*L. gmelinii*	H	ES	H	0–1450	5–10

Table 2. Continued

Families	Taxa	Life form	Chorotype	Ecotype	Altitude (m above sl.)	Flowering time (months)
Plumbaginaceae	*L. graecum* var. *graecum*	H	M	H	sea level	5–7
Plumbaginaceae	*L. ocymifolium*	H	M	H	sea level	5–7
Plumbaginaceae	*L. sieberi*	CH	M	H	0–5	5–7
Plumbaginaceae	*L. sinuatum*	H	M	H	0–100	5–7(10)
Plumbaginaceae	*L. virgatum*	H	M	H	0–20	6–10
Poaceae	*Aeluropus littoralis*	C	IM	PM	0–1200	5–10
Poaceae	*Aira elegantissima* var. *elegantissima*	T	M	PM	0–300	4–5
Poaceae	**Alopecurus myosuroides* var. *latialatus*	T	M	H	sea level	5
Poaceae	*Ammophila arenaria* subsp. *arundinacea*	C	M	PM	sea level	6–8
Poaceae	*Briza maxima*	T	IM	X	0–320	4–5
Poaceae	*Bromus arvensis*	T	IM	H	0–2900	6–8
Poaceae	**B. psammophilus*	T	M	PM	sea level	6
Poaceae	*B. rubens*	T	IM	PM	0–1000	3–6
Poaceae	*B. tectorum*	T	CSM	PM	0–2000	3–6
Poaceae	*Catabrosa aquatica*	C	IM	H	0–2600	5–8
Poaceae	*Corynephorus divaricatus*	T	M	X	0–1100	4–6
Poaceae	*Crypsis aculeate*	T	IM	PM	0–1510	6–10
Poaceae	*Cutandia dichotoma*	T	IM	PM	sea level	5
Poaceae	*C. maritima*	T	M	PM	sea level	5–6
Poaceae	*Cynodon dactlylon* var. *dactylon*	C	IM	PM	0–1830	4–9
Poaceae	*Elymus factus* subsp. *farctus* var.	C	M	PM	sea level	6–8
	farctus					
Poaceae	*Hordeum marinum* var. *marinum*	T	IM	PM	0–830	5–6
Poaceae	*H. marinum* var. *pubescens*	T	ES	H	0–100	5–6
Poaceae	*H. murinum* subsp. *glaucum*	T	IM	X	0–1750	4–7
Poaceae	*Imperata cylindrica* subsp. *cylindrica*	C	IM	PM	0–760	4–7
Poaceae	*Lagurus ovatus*	T	M	PM	0–50	4–6
Poaceae	*Lolium rigidum* var. *rigidum*	T	IM	H	0–1850	4–7
Poaceae	*Parapholis filiformis*	T	M	PM	sea level	6
Poaceae	*P. incurva*	T	IM	H	0–100	4–7
Poaceae	*P. canariensis*	T	IM	H	0–1000	5–7
Poaceae	*Phleum exaratum* subsp. *exaratum*	T	IM	H	0–2300	5–7
Poaceae	*Phragmites australis*	C	ES	H	0–2400	8–10
Poaceae	*Poa bulbosa*	C	IM	X	0–3000	5–7
Poaceae	*P. trivialis*	C	IM	H	0–2210	5–8

Table 2. Continued

Families	Taxa	Life form	Chorotype	Ecotype	Altitude (m above sl.)	Flowering time (months)
Poaceae	*Polypogon maritimus* subsp. *maritimus*	T	ES	PM	0–400	5–6
Poaceae	*P. monspeliensis*	T	ES	H	0–1200	4–8
Poaceae	*Prapholis incurva*	T	IM	H	0–100	4–7
Poaceae	*Puccinellia festuciformis*	H	IM	H	sea level	5–6
Poaceae	*Sporobolus virginicus*	C	IM	H	0–950	4–10
Poaceae	*Triplachne nitens*	T	M	PM	sea level	5
Poaceae	*Vulpia fasciculata*	T	M	PM	0–20	5–6
Polygonaceae	*Polygonum aviculare*	T	CSM	PM	0–700	7–11
Polygonaceae	*P. equisetiforme*	H	IM	X	sea level	6–9
Polygonaceae	*P. maritimum*	H	IM	PM	sea level	6–11
Polygonaceae	*Rumex bucephalophorus*	T	M	X	0–150	2–5
Primulaceae	*Anagallis arvensis* var. *arvensis*	T	IM	PM	0–1400	4–9
Primulaceae	*Glaux maritima*	C	IM	H	0–1720	5–8
Primulaceae	*Lysimachia atropurpurea*	T	M	R	0–1000	5–10
Primulaceae	*Samolus valerandi*	H	CSM	H	0–900	5–9
Ranunculaceae	*Ranunculus marginatus* var. *marginatus*	T	CSM	H	0–850	3–6
Rosaceae	*Rubus sanctus*	CH	IM	X	0–1250	6–8
Rubiaceae	*Oldenlandia capensis*	T	IM	H	sea level	9
Scrophulariaceae	*Rhamphicarpa medwedewii*	T	IM	H	sea level	8
Scrophulariaceae	*Scrophularia canina*	CH	M	H	0–1500	4–7
Scrophulariaceae	*Verbascum sinuatum* var. *adenosepalum*	H	M	PM	0–1100	5–10
Scrophulariaceae	*V. sinuatum* var. *sinuatum*	H	M	PM	0–1100	5–10
Solanaceae	*Solanum alatum*	H	IM	X	0–1350	6–11
Tamaricaceae	*Tamarix hampeana*	P	IM	H	–	4
Tamaricaceae	*T. parviflora*	P	M	H	0–300	3–6
Tamaricaceae	*T. smyrnensis*	P	IM	H	0–1000	4–8
Tamaricaceae	*T. tetrandra*	P	IM	H	0–1300	5
Thymelaeaceae	*Thymelaea hirsuta*	CH	M	X	sea level	9–12
Verbenaceae	*Phyla nodiflora*	CH	IM	PM	sea level	4–8
Verbenaceae	*Phyla nodiflora*	CH	IM	PM	sea level	4–8
Verbenaceae	*Verbena officinalis*	CH	IM	X	0–1800	6–8
Zygophyllaceae	*Tribulus terrestris*	T	IM	X	0–1200	6–9

IT: Irano-Turanian, **ES:** Euro-Siberian, **M:** Mediterranean, **IM:** Imperfectly known, **Ss:** Sahoro-Sindian, **CSM:** Common, **ID:** Indifferent, **H:** Hemicryptophytes, **CH:** Chamaephytes,**T:** Therophytes, **C:** Cryptophytes,**P:** Phanerophytes, **PM:** Psammophytes, **H:** Halophytes, **X:** Xerophytes, **R:** Ruderals, ***:** Endemics

During the present survey 213 taxa belonging to 37 families were recorded to show a distribution along the Turkish Mediterranean coast (Tab. 2). The four major families embodying the highest number of taxa are Poaceae (26 taxa), Asteraceae (21 taxa), Fabaceae (23 taxa) and Chenopodiaceae (19 taxa) (Tab. 2). A similar situation is seen in the neighbouring Mediterranean country of Greece [17]. The genera *Limonium, Plantago, Tamarix, Chenopodium, Juncus, Bromus* and *Astragalus* have the highest number of taxa (Tab. 2). The distribution of different ecological forms is as follows; halophytes (85 taxa), psammophytes (67 taxa), xerophytes (47 taxa) and ruderals (14 taxa) (Tab. 2). Phytogeographically, 51.17 % of these taxa are imperfectly known, 35.69 % Mediterranean, 6.57 % Cosmopolite, 5.16 % Euro-Siberian, 0.94 % Sahoro-Sindian and 0.47 % Irano-Turanian. The life form spectrum reveals that 103 taxa of the coastal plant cover are therophytes, 39 taxa are hemicryptophytes, 36 taxa are chamaephytes, 30 taxa are cryptophytes and 5 taxa are phanerophytes (Tab. 2). Out of the 213 taxa, 13 are endemics. The red data book of Turkish plants reveals that 13 endemics and 10 non-endemic taxa from the coastal plant cover are in danger of becoming extinct [18].

Soils and groundwater

The coastal zones abound in sand dunes and saline habitats. Soils and groundwater samples collected from the coastal zone cities were subjected to a detailed physical and chemical analysis using the methods outlined in detail in [19, 20].

In summers, soils are highly saline with E.C values ranging between 30.53 and 67.95 dS/m. The pH varies between 7.46 and 9.09, being strongly alkaline to slightly alkaline, the dominant cation is sodium (367.1–714.32 me/lt), the dominant anion chloride (338.23–871.67 me/lt) and boron values lie between 0.57 and 2.729 ppm. E.C values in the groundwaters during summer lie between 31.17 and 79.98 dS/m, being highly saline, pH is 7.01–7.51, being neutral to slightly alkaline. The dominant cation is sodium (281.76–795.7 me/lt), dominant anion is chloride (329.7–952.52 me/lt) and the boron content is very high (1.4–3.52 ppm).

E.C values in the soils during winter vary between 3.95 and 36.95 dS/m, being saline, medium-saline and highly saline, pH being 7.67–9.10 and these are strongly alkaline, medium alkaline to slightly alkaline; the dominant cation is sodium (38.7–415.3 me/lt), the dominant anion is chloride (25.0–451.5 me/lt) and the boron values are higher (1.47–4.5 ppm). In the groundwaters, E.C values in winter lie between 7.36 and 85.08 dS/m, being slightly saline to highly saline. The pH varies between 7.02 and 8.28 and these are neutral, slightly alkaline, medium alkaline; the dominant cation is sodium (70.2–976. me/lt), the dominant anion is chloride (61.2–1083.15 me/lt) and the boron content is very high (0.98–4.75 ppm).

E.C values in the soils during spring are in the range of 8.57–49.48 dS/m, being slightly saline to highly saline; the pH lies between 7.72–9.03, being strongly alkaline, slightly alkaline to medium alkaline; the dominant cation is sodium (79.15–593.02 me/lt), the dominant anion is chloride (67.45–635.07 me/lt) and the boron content is high (1.9–5.3 ppm). The E.C values in the groundwaters in spring lie between 17.85 and 93.59 dS/m, these are medium saline to highly saline; the pH lies between 7.11 and 7.92, being slightly alkaline to medium alkaline; the dominant cation is

sodium (102.6–1147.2 me/lt), the dominant anion is chloride (107.2–1223.12 me/lt) and the boron content ranges between 0.88 and 5.02 ppm.

E.C values in the groundwaters during autumn vary between 31.04 and 69.74 dS/m and all samples are highly saline. The pH of the samples lies between 7.49 and 8.25, being medium alkaline to slightly alkaline. The dominant cation is sodium (331.5–745.45 me/lt), dominant anion is chloride (295.8–1004.25 me/lt) and the boron values lie between 0.9–3.85 ppm.

Some investigations on the salinity–alkalinity problems of the Turkish Mediterranean coastal zone cities have been carried out earlier [21]. The saline and sodic soils in the Mediterranean region cover an area of 560,000 ha. In Muğla, Antakya and Antalya states, these vary between 5,000 and 50,000 ha; in Izmir, Aydın and İçel between 50,000 and 100,000 ha, and in Adana over 100,000 ha. The saline areas are greater in size than its neighbouring Mediterranean country Syria (532,000 ha).

Land degradation activities

The biotic pressures significantly damaging the coastal zones are demographic developments and urbanization, summer houses, exploitation for tourism and recreation, salinity–alkalinity problems, industrial activities, reclamation of land from dunes and wetlands and sand extraction [4, 22]. A large number of marshes on the coast have been changed into touristic resorts or agricultural areas. The climate, soils and vegetation interact strongly with such activities. There has been a steady migration during the last few decades towards the socio-economically developed coastal zones such as the Mediterranean and Aegean regions, leading to overpopulation and heavy urbanization in the area [4]. Both these regions have experienced a high tourism activity. The attractive coastal cities from Kuşadası to Alanya hosted 11.6 million foreign tourists in 2004. This, together with the illegal invasion of the coastal zone for cultivation, heavy pressure of pollutants brought from inland areas, untreated wastewater resulting from urbanization and industry and summer houses has resulted in the degradation reaching a stage where negative impact is currently limited but imminently significant [23, 24]. These areas use high amounts of freshwater, pesticides and fertilisers resulting in the deterioration of this ecosystem.

Management strategies

Throughout history, the coastal zones were exploited and disputed by their inhabitants. The situation in the Mediterranean phytogeographical region of Turkey is no different. But fortunately today people have started to realize that it is necessary to stop abusing this fragile environment. However, for the management of these areas, a broad range of information spanning different fields is needed. The dynamic management response will be possible by a close monitoring of the status of coastal ecosystems, quality of the habitats and economic indicators [23–25]. For this purpose, sound information on plant diversity, vegetation cover, habitat types, and locations of species-communities should be collected and land cover maps should be prepared to note the status of habitat deterioration caused by climate fluctuations and by human impact [26–28]. The plant diversity representing various life forms together with species richness and other biodiversity indicators will help in the de-

termination of site quality as presented in this work. Salinization and alkalization problems in the coastal states are also increasing [21]. The percentage of soils with high carbonate concentrations is very high and soils with a pH value in excess of 8 are 4.7 %. An ecologically sound way to cope with this situation would be the use of halophytic taxa in such areas. The halophytes could be evaluated as potential agricultural crops by growing them on saline soils, in particular along the coastal parts where seawater is available for irrigation, instead of destroying them as a wasteful group of plants. However, this will rely on a high degree of salt tolerance, not only of the perennial species used to lower a saline water table, but also of the crops to follow, as some salt will remain in the soil. The species like *Salicornia europaea* and *Suaeda maritima* may be a good choice for such areas. In fact there is a great potential existing in the halophytic plant cover for consumption as well as amelioration of the degraded lands along the coastal zones [10, 26]. The coastal zone plant cover is mainly composed of psammophytes and halophytes. They can be used for biological desalination and reclamation of saline–alkaline habitats [7]. Remote sensing and GIS (Geographic Information Systems) should be used in the mapping and monitoring of coastal ecosystem [24, 28]. A multidisciplinary program following a science based approach for methods, standards, data collection, research networks and development of land use models incorporating natural/human induced factors should be started for the evaluation of ecologically sustainable economic productivity potential of the coastal zone areas. The overlapping of responsibilities among different authorities in the region should immediately be solved. These measures can be used for designing conservation policies. For this purpose both national decision-makers and non-governmental organizations, as well as communities living in the area, should join hands to overcome this problem.

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Biosaline Agriculture and Salinity Tolerance in Plants
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Saline and alkaline vegetation of NE Africa and the Arabian peninsula: An overview

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Introduction

The saline and alkaline vegetation of NE Africa and Arabia is influenced by the diverse geomorphology of the coastline and inland arid plains, and by the distribution patterns of tropical and extra-tropical plant species. A zonation of species from the sea or lake landwards is distinct and is determined by the declining influence of salinity and/or alkalinity, duration and degree of inundation, and structure of the substrate.

One of the most prominent features of saline or alkaline vegetation is that it is impoverished in the number of species and hardly exceeds 300 taxa. The main plant families, to which these belong, include Acanthaceae, Amaranthaceae, Asteraceae, Chenopodiaceae, Fabaceae, Plumbaginaceae, Polygonaceae and Zygophyllaceae. As the area is arid with high daytime temperatures, the majority of the species present show C4 photorespiration pathway thus allowing them to survive in these high temperatures, intense light and low moisture conditions.

Another feature of saline or alkaline vegetation is that much of it is composed of monospecific stands though mosaics with two or three species are also found. The majority of the species are obligate halophytes, but salt tolerant and salt secreting species such as *Sporobolus*, *Aleuropus*, *Limonium* and mangroves are also present. In tropical East Africa the arid and saline species are predominantly annuals while in Arabia they are chiefly succulents and hemicryptophytes.

In this paper I review the halophytic and glycophytic vegetation of NE Africa and Arabia, describe the main plant communities and the typical vegetation zonation patterns, significant differences in the tropical and extra-tropical flora and vicariance in the Arabian halophytic flora.

Geographical area

The study area consists of the north eastern part of Kenya, NE and coastal south-east Yemen, coastal and inland Oman, the Saudi Arabian desert region, and coastal regions of the UAE, Qatar and Kuwait.

In tropical East Africa halophytic and glycophytic vegetation is limited mostly to lake basins in the Eastern Rift (mainly in the Kenyan Rift Valley) and a few coastal

areas. A relatively large inland salt lake lies in north Kenya, west of Lake Turkana, associated with the Chalbi desert (a former lake).

The 8,000 km long coastline of the Arabian Peninsula shows a diverse topography of low sandy coastal dunes, flat, silty-saline depressions, and cliffs and littoral mountains. Climate throughout the Arabian Peninsula is arid with very little rainfall [46], and therefore there are large inland areas of high salinity with obligate halophytic vegetation.

The present review is based on publications by various authorities on tropical East Africa and the Arabian Peninsula, and my own research in the Sultanate of Oman. Various aspects of the vegetation of tropical East Africa has been described by Bogdan [1], Vesey-FitzGerald [2], Gillett et al. [3], Knapp [4], Clayton et al. [5], Lind and Morrison [6], and White [7]. A comprehensive account of the vegetation of the Arabian Peninsula is given in Ghazanfar and Fisher [8] with details of the coastal and halophytic vegetation by Deil [9 and references therein], Mandaville [10 and references therein], Ghazanfar [11, 12 and references therein].

Halophytic vegetation of tropical East Africa

The desert vegetation of tropical East Africa (northern Kenya) falls under the Somalia-Masai regional centre of endemism which includes NE Uganda (Karamoja), most of Kenya between the Highlands and the coastal belt, and the dry lowlands of north and central Tanzania. The vegetation is characterised by an *Acacia–Commiphora* deciduous bushland and thicket, and grassland. Two genera, *Drake-Brockmania* and *Dasysphaera* are endemic to this floristic region.

The dry and halophytic vegetation is found primarily in the Chalbi desert, which is sparsely vegetated at the edges where there is subsurface water flow from springs from the surrounding mountains. Due to extensive evaporation the entire area is hyper-arid, and after seasonal rains and flood, the soil becomes highly saline supporting a few halophytic grasses which grow near runnels and water outlets.

In the surrounding plains and parts of the Chalbi desert the most extensive vegetation type is the semi-desert annual grassland. The main halophytic grass is *Drake-Brockmania somalensis*, a mat-forming annual, spreading by stolons. It occupies seasonally flooded places in silty and saline soils. *Drake-Brockmania somalensis* is distributed from Tanzania through to NE Africa (Sudan, Somalia, Ethiopia; excluding Uganda); it is also found on the Farasan Island (Saudi Arabia) in the Red Sea [13].

By far the most common and widespread in the desert and semi-desert are the annual grasses, *Aristida adscensionis* and *A. mutabilis*. These occupy the driest areas, extending up to elevations of 1,000 m on the drier parts of the surrounding hills. They occur on poor, shallow soils and during periods of drought may be absent for as long as the drought lasts [7]. Associated with these are a few species of subshrubs which form the perennial woody components of the sabkhat. Dominant among these are *Duosperma eremophilum* (Family Acanthaceae) and *Indigofera spinosa* (Family Leguminosae); the former found on relatively moister soils, and the latter on the drier soils.

Among other shrubs, *Lagenantha nogalensis* (Family Chenopodiaceae) a succulent that is tolerant of gypsophilous soils, forms almost pure stands on white calcare-

ous soils of the old Chalbi lake bed; *Volkensinia prostrata* (Family Amaranthaceae) occurs on saline or alkaline soils at the margins of Lake Turkana and at the edges of the Chalbi desert [7].

Only a few trees or large shrubs are associated with arid and saline soils. These are usually stunted, with *Acacia reficiens* as the dominant species.

The main species around the saline lakes in Kenya and Uganda are *Cyperus laevigatus, Sporobolus spicatus* and *Dactyloctenium* spp. The vegetation of the grassland on the flats close to Kiboko river is described by Bogdan [1], which shows different dominant species inhabiting soils with different concentrations of salts: thus in slightly alkaline conditions the grass cover is mainly *Cenchrus ciliaris,* but as alkalinity increases *Chloris gayana* becomes dominant. With increasing moisture and alkalinity *Sporobolus robusta* becomes the dominant species and where soils are shallow, *Cynodon dactylon* appears, and in highly alkaline soils that are waterlogged in the rainy season, *Sporobolus spicatus* takes over and forms dense growth. In the flat valleys in the drier parts of Tanzania, such as the Pangani river valley, where the water is saline, the flood plains are dominated by grasses *Sporobolus robusta*, and shrubs such as *Suaeda monoica*, *Sesbania sesban, Salvadora persica* and *Triplocephalum holstii*.

The vegetation of the Lake Rukwa basin in Tanzania, which is chiefly grassland has been described by Vesey-FitzGerald [2]; this is summarised here: the vegetation can be divided into three zones from the fringe of the lake to the lake bed itself. The edges of the lake are occupied by almost pure stands of *Sporobolus robusta*. This species does not grow in water, but colonises the alkaline soils on the beach of the lake. The shallowly flooded alkaline swamp present on the extensive flat lake bed is vegetated by a single species *Diplachne fusca*. This is a rhizomatous perennial rooting and branching from the lower nodes forming dense mats over large areas. On the lake bed, the alkaline flats are occupied by two species: when the lake is dry *Spobolus spicatus* grows associated with *Psilolemma jaegeri*; when the flats are flooded with the highly alkaline water, *Psilolemma jaegeri* takes over and replaces *Sporobolus*.

Other saline and alkaline patches of vegetation exists around Lake Amboseli in Kenya, where the major vegetation type is the *Commiphora–Acacia* shrubland, and the saline and alkaline plains are dominated by *Suaeda monoica* and *Salvadora persica*. Alkaline grassland dominated by *Sporobolus spicatus* is also present around Lake Manyara. On the coast *Suaeda monoica* may form monospeciefic stands at the edges of the barren sand flats, while *Arthrocnemum indicum, Sporobolus virginicus* and *Suaeda monoica* grow on slightly elevated soil. *Salicornia pachystachya* may form carpets in summer. Other species include *Sesuvium portulacastrum* in the more moist areas.

The dry *Acacia–Commiphora* shrubland continues through from NE Africa to SW Arabia, and in Somalia the associated species on the the coastal plain include *Aerva javanica*, *Jatropha pelargoniifolia*, *Farsetia longisiliqua*. On gypsophilous soils succulent euphorbias (*E. coluumnaris*, *E. sepulta*, *E. mosaica*), *Dorstenia gypsophila*, *Pelargonium cristophoranum* are present [14].

Halophytic vegetaion of the Arabian Peninsula

The main species in saline habitats are mostly perennial succulents, subshrubs and stoloniferous hemicryptophytes. Among the annuals, succulents species such as *Bienertia cycloptera* and *Zygophyllum simplex* are rare. The most common coastal and salt tolerant species are *Arthrocnemum macrostachyum*, *Halocnemum strobilaceum*, *Halopeplis perfoliata*, *Limonium* spp, *Salsola* spp, *Salicornia europaea*, *Seidlitzia rosmarinus*, *Suaeda* spp, *Zygophyllum* spp; the grasses and sedges *Aeluropus lagopoides*, *Juncus rigidus*, *Odyssea mucronata*, *Sporobolus spicatus*, *S. consimilis*, and mangroves *Avicennia marina*.

The typical plant communities of the coastal vegetation have been described from the Gulf of Aqaba [15–17], the Red Sea coast north of Jeddah [18], Tihama Coast [19], the Gulf of Aden [20–22], the Hadhramaut coast at Felek, east of Mukalla [22], the coastal, inland sabkha, and saline and brackish water vegetation of Oman [12, 23–26], the coastal vegetation of the islands of Masirah and Shagaf 27], vegetation of the Qurm Nature Reserve near Muscat [28, 29], coastal vegetation near Dubai [30], halophytic vegetation of Qatar [31–37], Bahrain [38–40], and Kuwait [41–43].

A summary of the typical vegetation of the Arabian Peninsula given here is taken from Deil [9]. For a detailed study see Deil [9 and references therein].

For the coastal vegetation, in the Gulf of Aqaba, generally the first zone consists of *Avicennia marina* on mud deposits followed by a *Limonium axillare* zone and then by a *Nitraria retusa–Zygophyllum album* zone. *Sueda monoica–S. vermiculata* zone can be seen near the shoreline in some locations. Where the coast is frequently inundated by the sea resulting in the top soil to be high in salt and the water table shallow (30–70 cm), *Arthrocnemum glaucum* is present. Where there is no inundation during high tide, a sterile zone is normally present followed by a *Suaeda pruinosa* zone. On the eastern coast of the Gulf of Aqaba a *Nitraria retusa* zone is present where the water table is at 100–140 cm, with the associated species *Zygophyllum album*, *Z. coccineum* and *Tamarix* spp. The last zone, a *Zygophyllum coccineum* zone, occurs on coarse textured sand with *Cyperus conglomeratus* and *Fagonia bruguieri* as associates. *Hyphaene thebaica* has its northernmost distribution near Eilat. The southernmost distributional limit of *Limonium pruinosum* is the southern part of the Gulf of Aqaba, replaced further south by *L. axillare*. A *Salvadora persica* open shrubland occurs on sand mounds and in alluvial fans where fresh water is close to the surface, and an open shrubland with *Nitraria retusa* and *Zygophyllum album* is present on aeolian sands where the groundwater is salty.

Along the Yemeni Red Sea coast northwards from Wadi Siham *Avicennia marina* zone is followed by a *Limonium cylindrifolium–Suaeda fruticosa–Limonium axillare* community which forms hummocks. A sterile sabkha is present, after which raised beaches above the high tide level are covered by *Atriplex farinosa*, *Zygophyllum hamiense, Aeluropus lagopoides* and *Halopyrum mucronatum*. Sand dunes towards the seaward side are colonised by *Suaeda monoica* and *Salsola spinescens*, and the inland dunes by *Odyssea mucronata*, *Jatropha pelargoniifolia* and *Leptadenia pyrotechnica*.

The southwestern corner of the Arabian Peninsula is characterised by the occurrence of a new coastal species, *Odyssea mucronata*, endemic to this part of Arabia. *O.*

mucronata is a clump-forming, spiny, rhizomatous perennial which colonises semi-mobile dunes and flat sandy areas. Depending on the depth of sand, an *Odyssea mucronata–Suaeda monoica* community can be distinguished on flat sandy layers overlying saline silts, and an *Odyssea mucronata–Panicum turgidum* community on deeper sand.

The Hadhramaut coast is situated in the transition zone from the southeastern to the southwestern vegetation type. This is seen from the *Cyperus conglomeratus* associations, where the Omano-Makranian element [44], *Coelachyrum piercei* and the Eritreo-Arabian element *Odyssea mucronata* are common members. The coastal vegetation shows a strong phytogeographical relationship with the coasts of northeast Africa. The species zones are: 1) coastal dunes colonised by sedges and grasses (*Cyperus conglomeratus*, *Halopyrum mucronatum*, *Odyssea mucronata*, *Coelachyrum piercei* and *Panicum turgidum*); 2) sandy-salty depressions colonised by the endemic *Urochondra setulosa* association, with the co-dominant *Arthrophytum macrostachyum*, *Limonium cylindrifolium* and *Crotalaria saltiana*; 3) clayey-salty, relatively wet areas colonised by monospecific stands of *Arthrophytum macrostachyum*; 4) sandy coastal plains colonised by the endemic *Anabasis ehrenbergii–Pulicaria hadramautica–Zygophyllum hamiense* association; 5) the karstic limestone plateau colonised by *Stipagrostis paradisea*, *Commiphora gileadensis* and *Euphorbia rubriseminalis*.

In the coastal vegetation of Oman four plant communities can be recognised: 1) A *Limonium stocksii–Zygophyllum qatarense* community in northern Oman where the coasts are mainly sandy and interspersed with rocky limestone headlands; 2) a *Limonium sarcophyllum–Suaeda aegyptiaca* community characteristic of rocky shores with narrow beach areas and a wide spray zone; 3) an *Atriplex–Suaeda* community characteristic of the vegetation of offshore islands, flat sandy beaches and coastal sabkhas (dominant and associated species are *Atriplex coriacea*, *A. farinosa*, *A. leucoclada*, *Arthrocnemum macrostachyum*, *Suaeda aegyptiaca*, *S. vermiculata*, *S. monoica*, *S. moschata* and *Halocnemum strobilaceum*), and a *Limonium axillare–Sporobolus–Urochondra* community characteristic of the vegetation of the southern coasts, with *Limonium axillare, Urochondra setulosa* and *Sporobolus* spp associated with several other species depending on coastal geomorphology; 4) coastal lagoons with *Sporobolus virginicus*, *S. iocladus* and *Paspalum vaginatum* as the main bordering species, and *Phragmites australis* and *Typha* spp forming the bordering reeds. In addition, *Avicennia marina* occurs throughout coastal Oman in discontinuous patches and over a wide range of water salinities [24].

On the Barr al Hikman Peninsula and the offshore island of Masirah, *Avicennia marina* is present in sheltered lagoons, a halophytic shrub community dominated by *Atriplex farinosa* and *Suaeda moschata* occurs on low coastal dunes which receive salt spray, and a *Halopyrum mucronatum–Urochondra setulosa* community occurs on more or less stabilised dunes. An *Arthrocnemum macrostachyum–Suaeda vermiculata* community occurs on the saline, silt plains and a *Limonium stocksii–Cyperus conglomeratus–Sphaerocoma aucheri* community on shallow sands.

A transect through the coastal dunes and sabkha in the UAE shows the typical dry haloseries within the Omano-Makranian region of the Arabian Gulf; four plant communities are present associated in the *Limonium stocksii–Zygophyllum qatarense*

vegetation complex: 1) the seaward dunes colonised by the *Cornulaca monacantha–Sphaerocoma aucheri* community (the Salsolo-Suaedetalia of Knapp [45]); 2) the landward dunes colonised by *Halopyrum mucronatum* (stabilising the sand), *Atriplex leucoclada* and *Suaeda aegyptiaca*; 3) salty depressions which may be temporarily inundated with seawater colonised by *Halopeplis perfoliata*; 4) an ephemeral, salt tolerant *Frankenia pulverulenta–Zygophyllum simplex* plant community growing in depressions with sandy overlays. The landward dunes, away from the influence of salt spray, are also dominated by *Cornulaca monacantha* and *Sphaerocoma aucheri*. They are associated here with glycophytic (i.e., non-halophytic) dune species such as *Panicum turgidum*, *Crotalaria persica*, *Lotus garcinii*, *Taverniera spartea* and *Indigofera intricata*. Similar also is the halophytic vegetation of Qatar, which along a transect from the mangrove zone to the sabkha plain, show a distinct floristic and edaphic gradient with the following zonation: 1) *Avicennia marina*, 2) *Arthrocnemum glaucum*, 3) *Halocnemum strobilaceum*, 4) *Juncus rigidus–Aeluropus lagopoides*. Associated species are *Zygophyllum qatarense*, *Halopeplis perfoliata* and *Anabasis setifera*.

In Kuwait *Salicornia europaea* grows on low, frequently inundated mud banks or along creeks, sometimes associated with *Aeluropus lagopoides* and *Bienertia cycloptera,* or with *Juncus rigidus* on the fringes of creeks. A *Halocnemum strobilaceum* community occupies the lower marshes along the shoreline with the seaward edge inundated very frequently by tides. A *Seidlitzia rosmarinus* community occurs further inland, followed by *Nitraria retusa* above the high tide mark dominating the middle marshes, and finally the *Zygophyllum qatarense* community on elevated, coarse sandy sites on the landward edge of the marsh. The salt marshes are fringed by non-halophytic communities such as the *Cyperus conglomeratus* community, the *Rhanterium epapposum–Convolvulus oxyphyllus–Stipagrostis plumosa* community and the *Haloxylon salicornicum* community, the latter covering most of the territory of Kuwait.

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Biosaline Agriculture and Salinity Tolerance in Plants
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Vegetation zones in the salty marshes of Central Anatolia and natural borders of agricultural usage (Turkey)

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Introduction

The region of Central Anatolia is rich in terms of lakes. These, Tuz Lake (Konya–Ankara–Aksaray), Seyfe Lake (Kırşehir) and Sultansazlığı (Kayseri) are surrounded by salty marshes. In the arid areas around these lakes, there are halophytic vegetation zones with changing physiognomy and floristic composition. In this study, edafic factors that cause the formation of vegetation zones, floristic composition of the zones and their situation from the point of view of agricultural usage are evaluated.

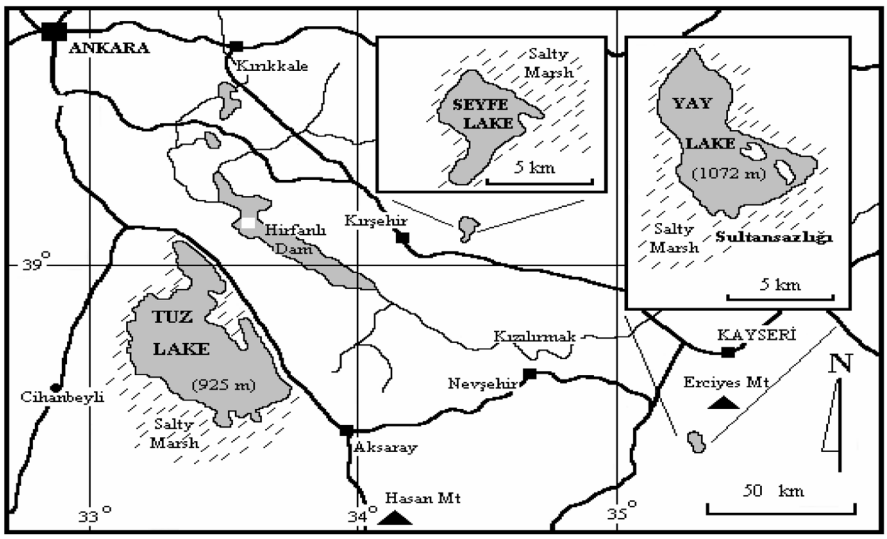

Figure 1. The most important salty marshes of Central Anatolia; Tuz Lake, Seyfe Lake and Sultansazlığı

Tuz Lake (925 m) is the biggest lake of Central Anatolia and is situated within the city borders of Ankara, Aksaray and Konya. Seyfe Lake (1,110 m) is in Kırşehir and Sultansazlığı is in the city borders of Kayseri (Fig. 1).

These lakes, which occupy the plains where drainage is spoilt in the region are under the influence of semi-arid or arid, very cold Mediterranean climate (Tab. 1). Annual average temperature changes range between 11.1–11.9° C. The highest temperatures are seen in July and August when the rain is minimum. The arid season, which begins at the end of June, continues for 4–5 months [1–3].

Table 1. The climate data of the closest meteorological stations to Tuz Lake, Sultansazlığı and Seyfe Lake

Areas and Stations	Altitude (m)	P (mm)	M (° C)	m (° C)	Q	PE (mm)	S	Preciptitat regime	Bio-climate
Tuz Lake (Aksaray)	980	347.4	299	13.4	36.4	36.5	1.2	Sp-W-A-S	Semi-arid, lower-very cold Mediterranean climate
Tuz Lake (Cihanbeyli)	969	308.0	296	−3.8	32.7	33.1	1.1	Sp-W-A-S	Arid, upper-very cold Mediterranean climate
Sultansazlığı (Develi)	1180	366.6	299	−4.4	37.4	31.2	1.0	Sp-W-A-S	Semi-arid, lower-very cold Mediterranean climate
Sultansazlığı (Yeşilhisar)	1150	274.8	309	−4.3	27.2	40.0	1.2	Sp-W-A-S	Arid, upper-very cold Mediterranean climate
Seyfe Lake (Kırşehir)	1100	351.5	352	−3.6	31.3	36.2	1	Sp-W-A-S	Arid, upper-very cold Mediterranean climate

P (mm): Mean annual precipitation, M (° C): Mean maximum for the hottest month, m (° C): Mean minimum for the coldest month, Q: Emberger's pluviometric quotient (2000. $P/M^2 - m^2$), PE: Summer rainfall, S: Emberger's index of xericity (S=PE/M), W: Winter, Sp: Spring, S: Summer, A: Autumn

The region of Central Anatolia forms the shape of a bowl, surrounded by mountains. Owing to its isolated nature, the region has a characteristic plant cover. In terms of phytogeography, it is included in the Irano-Turanian region and it forms a province called 'Central Anatolian' [4]. The salty marshes in the region constitute the richest areas of this province in terms of endemism (especially Tuz Lake). The first studies of the terrestrial salty marshes of Central Anatolia are related to halophytic communities of Tuz Lake and Konya plain [5, 6]. Thanks to the detailed phytosociological studies that have been realized in the last years, syntaxonomy of halophytic vegetation of Central Anatolia has been determined [7–10].

The region of Central Anatolia has a favorable topography for agriculture. This situation causes the region to be under intensive anthropogenic influence. Because of the fast increase in population increase and technological developments during the last 60 years, many pasture regions have been converted into farmland. Salty marshes

have suffered most from these activities. *Artemisia santonicum* L. communities that constitute the most outer zone of these areas are known to have spread in much larger areas before the agricultural activities became dense [11].

Recently, it has been seen that salty marshes are very rich in the diversity of species and it should be protected more effectively. However, it is not easy to draw borders between the areas that will be protected and that will be opened to agriculture. In the shaping of the borders, economical and political desires of human bodies are more diagnostic. Consequently, the borders of protection areas are kept as limited as possible. As the floristic and faunistic richness decreases over time, the faults that were made have been noticed and broadening the borders in many areas that have been protected today is an obligation.

Materials and methods

The results of the analyses of the soil samples that belong to the zones identified in the base of Yay Lake (Sultansazlığı, Kayseri), phytosociological studies made on salty marshes of Central Anatolia and observation of the authors constitute the material of this study. The soil samples were collected on 29 August 2004 and dried in shadow and sieved by a 2 mm sieve. Solving and reading processes are made according to [12].

Results

In the salty marshes, vegetation is formed by mixed zones. The most important factor shaping these zones is soil salinity and the salt tolerance limits of species. It means that the existence of the species in any zone is not a coincidence. Therefore, floristic composition of any zone gives us very important information about formations of the edaphic properties of that zone.

The zone formation of halophytic species, depending on their tolerance limits, is a basic property that can be observed from their first development. Although this is already known, it will be useful to show it again here. Yay Lake is the biggest lake in Sultansazlığı (Kayseri). The water of Yahyalı and Kovalı streams which feed this lake is accumulated in dams and used for agriculture. The amount of water in the lake has decreased over the last 5 years as the result of intensive agricultural activities. In the drying part of the lake, primary development of the vegetation (= halosere) has started. These areas are in the aggregation phase and have three zones. Each zone consists of single and dominant species. Although sometimes different species enter the zones, this situation doesn't affect physiognomy. The dominant species that form the zones in halosere, and analysis results of soil samples taken from these zones, are given in Table 2. According to results, the main cations and anions that cause the salinity of the soil are Mg^{2+}, Na^{+}, Ca^{2+}, K^{+}, Cl^{-}, ${SO_4}^{2-}$ and total ${CO_3}^{2-}$. As the amount of these increase, salinity increases. In Yay Lake, *Salicornia europaea* L. takes place in the first zone where these factors are in the highest density. These values decrease gradually in the second and third zones. Depending on this, there is

Salsola macera Litw. in the second zone and *Petrosimonia nigdeensis* Aellen in the third zone. These findings reveal the basic principals of simple but necessary zone formation.

Table 2. Soil analyzes results belonging to primary vegetation zones developed in the base of Yay Lake

Zones	Dominant species		Cations (μgg^{-1})				Anions (mgg^{-1})		
		pH ▼	Mg^{2+} ▼	Na^{+} ▼	Ca^{2+} ▼	K^{+} ▼	Total $CO_3{}^{2-}$ ▼	Cl^{-} ▼	$SO_4{}^{2-}$ ▼
1	*Salicornia europaea*	8.43	4,415	4,349	124,800	4,265	453	8.40	2.85
2	*Salsola macera*	8.35	4,304	3,343	64,438	3,999	428	5.29	1.89
3	*Petrosimonia nigdeensis*	8.20	4,282	2,406	55,384	3,359	414	3.34	1.42

The beginning of the climax vegetation seen in Central Anatolia is no doubt similar to the example of Yay Lake. These zones of vegetation have different characteristics not only in terms of physiognomy but also floristic composition and endemism.

1. Zone

In the inner zone where the salt density is maximum in Tuz Lake, Seyfe Lake and Sultansazlığı, in the first zone, *Salicornia europaea* L. and *Halocnemum strobilaceum* (Pall.) M.Bieb. are dominant (Fig. 2). The general cover in the zone changes between 10–80 % depending on salt density. The width differs according to the slope and the salt concentration. The zone is rather narrow in the regions with high slopes, but it can continue kilometers in the regions with very small slopes. Besides, this zone is observed in more hollow and, therefore, saltier areas in the other zones. Because of its high salt concentration, it is quite poor in terms of floristic composition. The number of species usually changes between 1 and 10 and it doesn't have any endemic

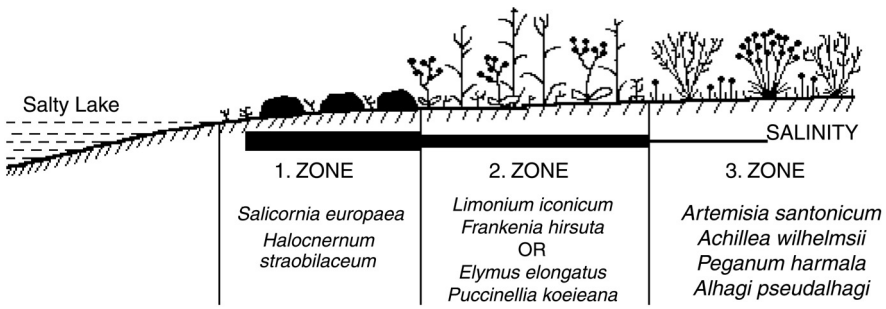

Figure 2. Sequence of vegetation zones depending on salinity in Tuz Lake, Seyfe Lake and Sultansazlığı

species. *Halocnemum strobilaceum* can also be seen in the second zone where the salt concentration decreased a little. In this situation floristic composition becomes a little richer and it can contain a few endemic species [5, 7–9, 13, 14].

2. Zone

Depending on the gradually decreasing salinity in the second zone, an instantaneous increase is observed in the number of species. In Central Anatolia this zone is seen with two different mixed physiognomies. The first physiognomy is dominant in the areas where water sources are not available. In this physiognomy where short succulent chaemophytes and hemicryptophytes are dominant, the number of species changes between 65–80 and endemism between 16–21 %. The most common species are *Limonium iconicum* (Boiss. and Heldr.) O. Kuntze, *Frankenia hirsuta* L., *Lepidium caespitosum* Desv., *Halimione verrucifera* (M.Bieb.) Aellen, *Salsola stenoptera* Wagenitz and *Puccinellia convoluta* (Hornem) P.Four, and *Halocnemum strobilaceum* which is also available in the first zone (Fig. 2). From these species, *Limonium iconicum*, *Lepidium caespitosum* and *Salsola stenoptera* are endemic for the salty marshes of Central Anatolia. In the areas where the water sources that feed the marsh are available, another physiognomy is dominant which is on the shape of tall herbaceous hemicryptophytes. Dominant species are *Elymus elongatus* (Host) Runemark subsp. *salsus* Melderis, *Puccinellia koeieana* Melderis subsp. *anatolica* Kit Tan, *Inula aucherana* DC., *Juncus maritimus* Lam., *Juncus heldreichianus* Marsson ex. Parl. subsp. *orientalis* Snog. and *Puccinellia convoluta* which is also available in the first zone. *Elymus elongatus* subsp. *salsus* is endemic for the salty marshes of Central Anatolia. In these areas the number of species changes between 45–60 and endemism between 26–32%. *Chenopodiaceae* and *Plumbaginaceae* are dominant in the first physiognomy and *Poaceae* and *Juncaceae* are in the other [5, 7–9, 14].

3. Zone (salty steppe)

In the salty marshes of Central Anatolia, the most outer zone is characterized by *Artemisia santonicum* L. (Fig. 2). This zone, called 'salty steppe', forms a border with the non-halophytic communities around. Succulent species are not seen very often in the zone. The individual cover of *Artemisia santonicum* is sometimes 70 %. The other common species of the zone are *Peganum harmala* L., *Alhagi pseudalhagi* (M.Bieb.) Desv., *Achillea wilhelmsii* K.Koch, *Noaea mucronata* (Forssk.) Aschers. and Schweinf. subsp. *mucronata* and *Apera intermedia* Hackel. In this zone the number of species changes between 70–95 and endemism rate between 17–23 % [5, 7, 10, 13].

In the first and second vegetation zones, usually true halophytes that have adapted to high salt concentrations grow. The vegetation period begins fairly late because these parts of the marsh are under water for a long part of the year. The upper surface starts to dry towards the end of July and it gets hard enough to be stepped on. In the salty steppes, vegetation period begins almost at the same time as the non-halophytic communities around. In this community, which is never under water in any period

of the year, there are usually no true halophytes. Non-halophytic species with a wide tolerance to salt and miohalophytes that can survive in a little salty environment are common in the zone.

The vegetation zones were compared in terms of floristic similarity by utilizing the phytosociological studies in the steppe areas in Tuz Lake and in the vicinity of Cihanbeyli which is a border to this area [9, 10, 15, 16]. According to this comparison, there is no floristic similarity between the first vegetation zone of Tuz Lake and Cihanbeyli steppe vegetation. In the second zone there is about 5 % floristic similarity in the areas where water sources are available and about 14 % in the areas where water sources are not available. Floristic similarity between salty steppes and Cihanbeyli steppe vegetation is about 38 %. These ratios show that the similarity to non-halophytic vegetation in the vicinity increases as the salinity decreases.

Artemisia santonicum communities shape the last zone in almost all salty marshes of Central Anatolia. They form a 'natural border' between true halophytic and non-halophytic communities. A big part of salty steppes have been converted into agricultural areas. In addition, the second and first zones have tried to be converted to agricultural areas by constructing drainage canals in some areas.

Discussion

Central Anatolia Region is the richest region of Turkey in terms of terrestrial salty marshes owing to its topographic structure. Out of these marshes, Tuz Lake is declared as a 'Special Environment Protection Area' and Sultansazlığı as a 'Nature Protection Area' and they are under protection. At the same time, these three areas are among the important 'watery areas' because they have a rich flora and they are the home of many bird species. Turkish Republic undertook to protect these areas by signing international treaties like RAMSAR (1971), BERN (1979) and RIO (1992).

However, the situation is not so positive today. In these areas, almost the entire salty steppe zone has been converted into agricultural areas. The other vegetation zones are under heavy threat. Recently, some salty marshes were almost killed by making new drainage canals or using water sources. When overgrazing, illegal hunting and collecting some plant species systematically for commercial purposes are added to the others activities. Hence, the end seems to be very close. One of the important dangers for the future of these areas is that people are not aware of the richness they have. Mentioning the salty marshes as 'deserts' in many regions shows the point of view about these areas.

Salinity is a serious threat in terms of productivity of the soil and it must be struggled with necessarily [17]. But when struggling with salinity, natural richness must be protected at the same time. Struggling with salinity in the present agricultural areas and trying to dry salty marshes which are quite rich in terms of biodiversity is difficult.

Today agricultural technology is quite developed and more products can be taken from a unit of area. These advances show that it is enough to make the present agricultural areas more productive instead of opening new agriculture areas. Struggling

with salinity must be intensified in the regions like Çukurova, Harran, Muş and Konya plains that have large agricultural areas.

Salty marshes should not be evaluated as the areas to struggle with salinity, but as the areas to be protected. When we look at the previous applications, the protection borders of salty marshes are usually determined as the second zones and even in some places, the first zones. Yet, first and second zones are the areas that must definitely be protected. Therefore, the protection borders in the salty marshes that are under protection in Central Anatolia should be reviewed again. Borders must begin from the salty marshes and if possible, the salty steppe zone with an apparent width must be also included in this border.

Today we should think and act more differently than we have in the past. We should not wait to lose the richness that we have in order to understand our faults. We must see this richness as a deposit and we should protect them with attention and we should try to transfer them to the future.

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Halophytes as cash crops for animal feeds in arid and semi-arid regions

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Introduction

Dry regions occupy more than one-third of the Earth's land area and are inhabited by approximately 16% of the World's population. In the arid countries, population increase, global climate change, a large number of the nomadic populations as well as over-use of renewable resources such as water has disturbed the sensitive ecological balance. Water resources in many of these countries are dwindling both in quantity and in quality. Such countries suffer from severe shortage of freshwater because of the dry climate and the irregularity of rainfall. Moreover, water resources of large parts of several countries are saline. The soils there are either naturally saline or have been salinized, mainly because of ill-conducted irrigation [1]. The potential utilization of halophytes grown under saline water irrigation would conserve freshwater and will enable crop production in marginal areas [2]. This article aims to draw attention to possible utilization of halophytes as cash crops, particularly in dry and saline ecosystems, and to focus primarily on the potential use of halophytes as animal fodder in arid and semi-arid regions.

Possibilities for halophytes utilization

There are more than 2,000 plant species registered as halophytes [3]. Several halophytes are presently used along seashores and inland salt marshes extensively. In salt affected areas, *Puccinellia* (*Puccinellia ciliata*), tall wheat grass (*Thinopyrum ponticum*) and Chenopods (e.g., various species of *Atriplex*, *Kochia* and *Bassia*) have been planted for the improvement of soil conditions due to their low transpiration rate, high efficiency of water utilization, drought and salinity resistance [4]. They get increasing attention today because of the steady increase of the salinity in irrigation systems in Mediterranean and subtropical desert countries where the increasing population has reached the limits of freshwater availability. Many halophytes have the potential to become cash crops for several reasons [2]:

- Halophytes for food such as *Aster tripolium* (salt aster), *Salicornia* sp., *Avicennia marina* and *A. germinant*.
- Halophytes for fodder such as many species of *Atriplex*, *Tamarix*, *Nitraria retusa* and grasses. All can provide good fodder for livestock and wildlife.
- Halophytes for wood such as *Tamarix* spp, and mangroves.
- Halophytes grown for chemicals: A variety of halophytes are collected for health and beauty products purposes or for tanning [3].
- Halophytes for landscaping: Fast growing plants can cover barren soils in a short time, e.g., *Batis maritima*, *Sesuvium portulacastrum* and *Atriplex* spp.
- Ornamental halophytes such as *Limoniastrum monopetalum* and *Aster tripolium.*
- Industrial raw materials: Growing halophytes for biomass is economically feasible only if additional elements, for compounds with especial value, are involved.
- Environmental protection: Many halophytic species are used for coastline protection such as *Spartina alterniflora*, *Spartina maritima* and *Avicennia marina.*

A number of considerations should be taken into account to assess the sustainability of a halophyte utilization system. The choice of the suitable crop depends on its local usefulness, the climatic zone, the adapted techniques for agricultural production and the ultimate objectives. The relevant aspects should be considered with the most important factor being water availability. However, economical prospects for halophytes can be evaluated when they are looked upon beyond their salt tolerance values. There are a number of other factors related to the plants establishment, e.g., productivity, water requirements, forage quality, etc., which could qualify the species for agricultural purposes. The following section briefly discusses the potential role of halophytes as feed materials.

Halophytes as feed resources

Shortage of feed resources is a common characteristic in arid and semi-arid regions and is considered the main constraint to improving livestock productivity. Therefore, intensive efforts have been directed to find alternative feed resources from saline habitats. Halophytes that are used as forage species will have better cash values if they have better forage quality. High palatability, digestibility and good nutritional value (high protein and lesser fiber, ash and oxalate contents) would significantly improve the forage quality.

Nutritive value of common halophytes

The biomass production, palatability and nutritive value of halophytes vary from area to area within the same region and from season to season depending upon several environmental factors [5]. Moreover, palatability and nutritive values vary significantly among halophyte species (Tab. 1). In general, most halophytes contain sufficient levels of crude protein, other essential nutrients that seems to cover the nutritional requirements of animals, particularly during the wet season [6, 7]. Fibrous

Table 1. Average value of chemical composition* and palatability index (PI)** of common halophytes (% on DM basis). [Sources: 6, 14]

Plant species	DM	CP	CF	EE	Ash	NEF	PI		
							Goats	Sheep	Camels
Halocn.strobil.ceum									
wet season	28.6	6.78	14.6	2.46	35.7	40.46	NP	NP	PP
dry season	37.8	4.22	19.6	2.16	42.5	31.52	NP	PP	PP
Zygophyllum album									
wet season	30.4	7.12	14.6	2.25	26.5	49.42	NP	NP	PP
dry season	39.3	6.30	16.2	1.63	28.5	47.31	NP	NP	PP
Tamarix mannifera									
wet season	57.4	7.64	16.1	2.21	26.0	47.97	PP	PP	FP
dry season	63.1	6.26	17.5	1.78	30.9	43.47	PP	NP	FP
Juncus acutus									
wet season	35.0	7.11	28.5	2.35	12.3	49.94	FP	FP	FP
dry season	42.3	6.00	33.5	2.05	14.0		PP	PP	FP
Salsola tetrandra									
wet season	45.0	9.73	12.4	2.61	30.1	45.10	FP	FP	HP
dry season	49.9	8.38	14.4	1.67	35.8	39.68	PP	PP	HP
Nitraria retusa									
wet season	14.6	9.10	12.8	3.01	16.2	58.87	HP	HP	HP
dry season	20.1	7.20	18.2	2.28	19.7	52.62	PP	FP	HP
Atriplex halimus									
wet season	42.1	9.21	18.7	3.20	31.4	37.49	FP	FP	HP
dry season	58.4	6.32	22.6	3.10	36.7	31.28	PP	PP	FP
Atriplex nummularia									
wet season	21.7	13.3	24.2	5.09	21.8	34.98	HP	HP	HP
dry season	39.8	10.2	28.0	1.90	26.7	33.81	HP	HP	HP
Suaeda fruticosa									
wet season	30.9	11.10	10.9	3.90	25.4	48.66	HP	HP	HP
dry season	48.3	8.40	13.7	3.00	30.3	44.60	FP	HP	HP

*DM: dry matter; CP: crude protein; CF: crude fiber; EE: ether extract, NFE: nitrogen free extract, **Palatability index : HP = Highly palatable, FP = Fairly palatable, PP = Poorly palatable

materials and ash contents are higher and increase while gross energy and protein contents are low and decrease with advancing maturity of the plants [8]. Therefore, most halophytes are nutritious during wet seasons and can sustain the maintenance requirements of animals. However, they are poor in summer and autumn (dry season) and need to be supplemented with other feed ingredients, particularly with energy feed resources [9, 10]. The chemical composition of *Atriplex* species, their nutritive values (digestible crude protein, DCP) and the total digestible nutrients (TDN) varied greatly [11, 12]. Salt marsh plants appear to contain sufficient concentrations of most major minerals (Tab. 2). They have no harmful effects even when high concentrations of such minerals are found in some plants [13]. However, some halophytes are deficient in sulfur and phosphorus [6]. No deficiency or toxicity of trace elements

was observed for most of the halophytic species [14, 13]. From data in Tables 1 and 2, it appears that the high ash content of most halophytes, in particular *Atriplex* spp, leads to high salt intake and consequently to a high water intake (average of 7 liters/day/head of sheep) and high urine output [7]. The high salt content of many halophytes has been shown to reduce the apparent energetic value and increased the flow of shrub materials through the digestive system [15]. The ash content of *Atriplex* spp is, therefore, an additional reason for mixing the shrubby materials with a low ash feed. The inclusion of a supplement, particularly of a soluble carbohydrate source, to the diet of sheep or goats eating halophytes showed an increase in the utilization of such forages. This is because it increases intake, digestibilities, growth rate and other animal performances [16]. Therefore, available sources of energy, phosphorus and sulfur should be supplemented to animals fed halophytes, particularly during the critical physiological status of animals [6, 9]. Performance of sheep and goats fed with *Atriplex nummalaria* and supplemented with crashed barley grains during the four seasons of the year was determined [8] and data presented in Table 3. It seems that DM, water, TDN, and DCP intakes, digestibility, nitrogen retention and weight changes were significantly affected by the season. Both sheep and goats utilized efficiently the supplemented *Atriplex* during the winter and spring seasons which was positively reflected by their higher body weight changes and all other criteria of feed

Table 2. Overall averages of major elements in *Atriplex* spp (% of DW) [Source: 13]

***Atriplex* spp**	**Na**	**K**	**Ca**	**P**	**Cl**	**Mg**	**S**
A. halimus	4.41	1.33	1.51	0.29	–	0.32	0.17
A. leucoclada	4.10	3.00	1.36	0.19	8.40	0.35	0.13
A. vesicaria	5.87	4.05	1.56	0.15	9.10	0.36	–
A. farinose	1.69	1.11	2.32	–	3.19	0.23	0.10
A. nummularia	4.15	3.19	1.26	0.11	7.80	0.30	0.20
A. canescens	3.90	2.08	1.43	0.16	–	–	0.18
A. semibaccata	3.32	1.35	1.29	0.11	0.10	0.33	0.25
A. glauca	6.30	1.60	2.14	0.13	–	0.56	0.11

Table 3. Utilization of supplemented *Atriplex nummularia* by sheep and goats [Source: 8]

Item	Sheep				Goats				F test
	W1	SP	SU	AU	W1	SP	SU	AU	
BW changes, g/d	55.6^{b}	77.8^{a}	23.2^{c}	11.1^{d}	49.4^{b}	66.6^{a}	17.1^{c}	-9.1^{d}	**
DM intake, g/d/Kg$^{0.75}$	52.0^{b}	59.8^{a}	47.7^{cd}	45.8^{cd}	53.7b^{b}	58.0^{a}	49.8^{c}	44.6^{c}	**
Water intake, ml/d/Kg$^{0.82}$	262^{c}	311^{b}	381^{a}	356^{a}	219^{d}	257^{c}	288^{bc}	269^{c}	**
N balance, mg/d/Kg$^{0.75}$	99^{b}	143^{a}	49^{c}	-25^{b}	86^{b}	126^{a}	39^{c}	-24^{b}	**
Digestibility, %DM	60.1^{a}	61.4^{a}	58.1^{b}	58.4^{b}	58.4^{b}	61^{a}	59.2^{b}	58.0^{b}	**
TDN, g/d/Kg $^{0.75}$	24.0^{b}	28.4^{a}	21.2^{bc}	19.0^{c}	25^{ab}	28.7^{a}	22.0^{bc}	18.7^{c}	**
DCP , g/d/Kg $^{0.75}$	3.4^{b}	5.0^{a}	2.8^{bc}	1.9^{c}	3.5^{b}	4.8^{a}	3.0^{bc}	1.9^{c}	**

W1, SP, SU, AU: winter, spring, summer and autumn, respectively, $^{**}P < 0.01$; Values on the same line with different superscript are significant

utilization. The opposite was found for the dry season (summer and autumn). These results reconfirmed earlier findings that additional feed supplements are required particularly during the dry seasons [6].

Enhancement of halophytes feed materials

Halophytes as individual forage materials have little prospect because long feeding periods are known to have adverse affects on browsing animals. Halophytic species vary in their palatability and acceptability to various animal species due to different factors [13]. Some of these plants can be fed to animals or grazed directly as fresh feed materials, particularly those that have high or moderate palatability and nutritious. Such plant species, i.e., *Atriplex* spp*, Nitraria retusa* and *Saudea fraticosa*, are always over grazed and disappear rapidly due to high grazing pressure of most animal species [6]. However, mixing halophyte forage with feed materials that are rich in protein or energy can significantly improve the feed nutritional value. A mix feed of dry grass and green *Atriplex* materials have been reported to increase the weight of goats in Pakistan [17]. Conversely, several halophytes are less palatable or unpalatable, producing a large biomass throughout the year. Utilizing such halophytes in arid countries is becoming necessary, particularly during the dry seasons or during prolonged periods of drought when other feed resources are scarce. These plants have some secondary metabolites, or so-called anti-nutritional factors, such as tannins, alkaloids, saponines, nitrites, etc., which negatively affect their use. Several approaches were undertaken to improve the utilization of such halophytic species through different processing treatments in order to improve their palatability and nutritive values [16, 18]. For instance, chopping can dramatically improve palatability of succulent species and enable a more efficient utilization of whole shrubs. Proper conservation such as hay making, haylage or ensiling processes of some halophytes could improve their utilization as good quality fodder. Results obtained by [16, 18, 19] for sheep and goats that were fed on various halophytic shrubs are summarized in Table 4. Different forms and combinations with other feed ingredients such as broiler litter, crushed date seeds, fodder beet and other forages were investigated. Ensiling process seems to improve the palatability of halophytes and their acceptability by sheep and goats. This might be due to the effect of anaerobic fermentation during the ensiling process on some anti- nutritional factors such as tannins and other phenolic compounds [20]. Additionally, the ash content and fiber materials are lowered [18]. Maximum intakes were improved greatly particularly for animals fed *Halocnemum strobilaceum* and *Atriplex halimus* silages (approximately 59 g DM/KgW$^{0.75}$) followed closely by *Tamarix aphylla* (averaged 43 g DM/KgW$^{0.75}$). Conversely, voluntary feed intake was increased by ensiling a mixture of some halophytic species, *A. halimus*, *H. strobilaceum*, *Tamarix mannifera* and *Zygophyllum album* with some agro-industrial byproducts such as ground date seeds, olive pulp, etc. [9]. Ensiling such halophytes with air-dried broiler litter improved their palatability and intake as compared to fresh or air-dried shrubs (Tab. 4). Animals fed these ensiled materials were in a positive nitrogen balance and tended to gain appreciable weight [18]. Similarly, [21] reported that sheep and goats consumed higher amounts

of silage comprised of a mixture of *Acacia saligna* and *Atriplex nummularia*, as compared with fresh or air-dried materials. Such silage, as sole basal diets, provided sufficient digested nutrients (TDN and DCP) to meet the maintenance requirements for sheep and goats. Feeding these silages to animals seemed to be more economic since feed costs decreased (about 30–50%) in comparison with conventional diets such as berseem hay [16, 18]. Recently, sex silages were formulated from mixing different natural and cultivated halophytic shrubs with other feed ingredients [22]. It appeared that silage 5, which contained ensiled cultivated (*A. nummularia* and *Acacia saligna*) and natural shrubs (*Tamarix mannifera*, *Zygophyllum album* and *H. strobilaceum*) with broiler litter, showed the highest level of crude protein (13.9 %). The CP contents of the tested silages, in particular silage 5, seemed to be reasonable to maintain the protein requirements of ruminants. The same authors also indicated that all silages attained higher ash contents which could be attributed to the inclusion of the natural halophytic shrubs. These silages contained higher levels of ash, Na and K concentrations which appeared to be in normal ranges and enough to cover the requirements of ruminants without an adverse indication.

Table 4. Intake of some halophytes in fresh, air-dried and ensiled forms [Sources: 12, 13, 19]

g DM/day/kg $W^{0.75}$	Sheep (S)	Goat (G)	S/G ratio
Fresh state			
A. halimus(AH)	19.4	15.8	1.23
H. strobilaceum (HS)	18.2	14.2	1.28
T. aphylla (TA)	9.69	8.72	1.11
T. mannifera (TM)	10.9	10.3	1.06
Z. album (ZA)	2.12	2.94	0.72
Air- dried state			
A. halimus	13.2	21.0	0.63
H. strobilaceum	9.45	8.23	1.15
T. aphylla	0.00	0.00	0.00
T. mannifera	5.12	4.75	1.08
Z. album	0.00	0.00	0.00
Silage state			
*AH+BL+MO**	59.9	59.7	1.00
HS+BL+MO	58.2	59.4	0.98
TM+BL+MO	46.5	42.9	1.08
TA+BL+MO	44.2	42.2	1.05
ZA+BL+MO	24.2	22.5	1.09
*Shrubs mixture*** *+GDS*	37.7	35.8	1.05
Shrubs mixture+ GDS+Urea	42.4	30.9	1.37
Shrubs mixture +FFB	18.0	11.2	1.61
Shrubs mixture +FFB +Urea	20.8	24.1	0.86

*BL: broiler litters; MO: molasses; GDS: ground date seeds; FFB: fresh fodder beet ** Shrubs mixture: AH+ HS+ TM+ ZA at 1:1:1:1

Utilization of halophytes by animal species

Small ruminants

Sheep and goats, as the most dominant animal species in arid regions, could be efficiently fed on halophytic forages as the main source of feeding materials. These animals should be supplemented with any available energy non-saline feed resources to enhance the utilization of halophyte feed materials. Crushed or ground barley grains, ground date seeds and molasses are commonly used as energy supplements for sheep and goats fed salt marsh plants. It was noticed that halophytes intake was affected by the levels of energy supplement; animals given barley grains showed higher forage consumption than those fed saltbush as a sole diet (Tab. 5). Barley grains improved the growth rate of sheep and goats fed on *Atriplex* spp and decreased water intake [8]. The daily amounts of 150 and 250 g barley grains/head were recommended for sheep fed on saltbush during all seasons [16, 23]. The effect of energy supplements on pregnant and lactating sheep and goats grazing the natural salt marsh plants in Sinai, Egypt were studied [6]. The grazing animals were supplemented with different levels of barley grains during two successive years. It appeared that both animal species could not sustain themselves on the natural ranges without supplements. Barely supplements improved the DM and nitrogen intakes of the ranges. The highest values were recorded for both sheep and goats given the supplement (100 % of the maintenance requirements). The performance of grazing dams during the pregnancy and lactation stages, in terms of body weight changes and milk yield, in addition to daily gain and weaning weight of their offspring were improved by the energy supplementation. Therefore, it was recommended to give additional supplements, particularly energy source, for productive animals grazing on halophytic pastures.

The effect of feeding six silages made from combinations of natural and cultivated halophytic shrubs plus a mixture of fresh cultivated shrubs of *Atiplex nummularia* and *Aacacia saligna* on the performance of six groups of sheep was investigated [22]. The results of body weight changes of these sheep varied significantly ($P < 0.01$)

Table 5. Average values of saltbush intake during the wet season

g/day/Kg $W^{0.75}$	Sheep	Goat	Sources
A. nummularia:			
Solely fresh	40.0	–	[23]
+ 150 g barley	59.8	58.0	[8]
+ 250 g barley	63.2	60.0	[16]
A. halimus :			
Solely fresh	19.4	15.8	[29]
Solely fresh	13.4	14.8	[16]
Solely dry	13.1	21.0	[29]
+ 100 g barley	26.4	27.6	[16]
+ 150 g barley	41.1	38.2	[16]

among treatments. Although the sheep used in this study were mature, some of them gained noticeable weight whereas others lost weight due to the fluctuations of the voluntary feed intakes pattern.

Camels

Camels are well adapted to arid and semi-arid regions, particularly to desert areas, where other animal species do not thrive and perhaps do not survive [4]. They have the capacity to utilize low quality feed resources as halophytes and convert them into animal protein and other products [24]. Camels could maintain themselves on natural ranges based mainly on halophytic plant species [4]. Some studies have been carried out on camels fed some halophytes in Egypt. 16 adult female camels were used to study the effect of energy level supplementation on the intake and utilization of *Atriplex nummularia* supplemented with yellow corn grain to cover none (group A or control), 20 % (group B), 40 % (group C) and 60 % (group D) of maintenance requirements of energy [24]. It is indicated (Tab. 6) that the voluntary intake of saltbush (*A. nummularia*) was significantly ($P < 0.01$) increased as a result of increasing the energy supplementation level which in turn was reflected on improving the body weight gain. The nutrients digestibility and nutritive values were also improved up to a level of 40 %. Studies on fattening camel on saltbush (*Atriplex halimus*) with different energy sources was conducted to evaluate growth performance, efficiency of feed utilization and carcass traits of male calves [24]. Daily intakes from DM, TDN and DCP were not affected by the experimental diets (Tab. 6). At the same

Table 6. Feed utilization of camel calves fed on various diets based on *Atriplex halimus* [24]

Item	**A**	**B**	**C**	**SEM**
Initial live weight (kg)	177.0	176.0	180.0	5.32
Daily gain (g/day)	750.0	732.2	692.2	0.03
Daily feed intake (kg/head):				
Concentrate mixture	2.68	–	–	–
Barley grains	–	2.47	1.87	–
Olive cake	–	–	0.85	–
Berseem hay	4.00	–	–	–
Fresh saltbush	–	13.00	11.00	–
Daily nutrient intake (kg):				
Kg DM/head	6.02	5.37	5.06	1.05
Kg TDN/head	3.75	3.36	3.65	0.69
Feed conversion:				
Kg DM/head	8.03	7.33	7.31	0.63
Feed cost /kg gain (US$)	0.76	0.38	0.36	–

Diet A = conventional concentrate + berseem hay (control diet), Diet B = ground barley + *Atriplex hamus* (fresh saltbush), Diet C = 75 % ground barley + 25 % olive cake + *Atriplex halimus* (fresh saltbush)

time, calves fed on saltbush and barley grains either only or with olive improved feed conversion in terms of kg TDN/Kg weight gain and Kg DCP/kg gain. Feeding camel calves on fresh saltbush resulted in appreciable reduction in feeding cost for production of one Kg body weight. Feeding camel calves on diets B and C reduced the cost of feeding by about 50 % and 53 %, respectively, compared to the control diet. It is clearly shown that fresh saltbush can be successfully and economically used in feeding camel calves in arid and semi arid zones.

Poultry

There is a shortage of feedstuffs used in poultry feeding in most of arid countries. Therefore, using non-traditional ingredients in poultry feeding can substantially contribute to solving this problem by decreasing the feeding cost. Research studies conducted in Egypt showed that *Atriplex* and *Acacia* leaf meals have a reasonable content of crude protein (16.20–19.95 %). Table 7 summarizes data on some nutrients and minerals in *Atriplex* leaf meal derived by some authors at the Desert Research Center, in Egypt. Data on *Acacia* leaf meal (ACLM) also reported a higher value of crude fiber, while ash content revealed a pronounced higher value in *Atripex* leaf meal (ATLM) as illustrated in [25]. The ATLM and ACLM could be successfully used as a non conventional feedstuff for poultry species as shown in Table 8. In this respect, [26] used ATLM safely in mash form at a level of 6 % and 12 % for growing rabbits and layer turkeys, respectively. Pelleted ACLM and ATLM were used for growing rabbits with up to 20 % and 25 % of the total diets [25, 27]. No effects on body weight gain, feed utilization and carcass characteristics were observed.

The impact of using such halophytic feed materials in poultry feeding greatly vary according to species, nutrient content of diet and some other environmental factors. Therefore, the recommended levels of ACLM and ATLM in poultry diets varied greatly. For example, ATLM was recommended at a level of 25 % in rabbit diets. This value was reduced to 6–12 % in growing layer turkeys. ACLM ranged from

Table 7. Chemical composition of *Atriplex* leaf meal (ATLM), on DM basis

Items	**Source[26]**	**Source[27]**
Crude Protein, %	19.95	18.89
Crude Fiber, %	2.43	6.26
Ether Extract, %	3.06	3.11
Nitrogen-free extract, %	40.91	41.33
Ash, %	22.05	20.19
Calcium, %	1.40	1.99
Phosphorus, %	0.40	0.24
Potassium, %	3.20	4.82
Sodium, %	5.90	8.96
Iron, ppm	0	21.0
Copper, ppm	78.0	28.0
Zinc , ppm	46.0	0

Table 8. Recommended levels of ATLM and ACLM percentages in poultry diets

Species	Author	ATLM	ACLM	Type of feeding
Growing rabbits	[30]	–	40	Wilting green leaves
Growing rabbits	[28]	–	15	Pellet
Growing rabbits	[25]		20	Pellet
Growing rabbits	[27]	25	–	Pellet
Growing rabbits	[26]	6	–	Mash
Layer turkeys	[26]	12	–	Mash
Broilers	[26]	–	6	Mash

15–30 % in a pelleted form for growing rabbits without detrimental effects on body weight gain and feed utilization, whereas this value decreased to 6 % for broiler diets. Increasing ACLM and ATLM in poultry diets was followed by an increase in total and in daily feed consumption. However, higher levels of ACLM and ATLM above the recommended values coincided with the decrease in body weight (daily gain and feed conversion). At the same time, nutritive value decreased due to the decreasing digestion coefficients of its nutrient content. Different levels of ACLM and ATLM had no significant effect on carcass characteristics and chemical components of meat in poultry. It is noted that increasing ACLM and ATLM in growing rabbit's diets resulted in an increased total and daily feed consumption [28, 25, 27]. This indicated that the inclusion of such ingredients in diets might increase their palatability. In this concern, [26] showed that inclusion of ATLM in layer and growing turkey's diets up to 6 % and 12 %, respectively, increased significantly the feed intake of birds as compared to that of the control group. The inclusion of ACLM and ATLM at higher levels caused a higher percentage of mortality (15 % at levels of 40 % ACLM and 30 % ATLM, respectively). This might be due to presence of some anti-nutritional factors in such halophytes (e.g., tannin, oxalate, etc.).

The lower costs of ACLM and ATLM are reflected on the feeding cost, net return and the values of economical efficiency. However, although ACLM and ATLM are non-traditional feedstuffs that could be used in poultry diets, attention must be given to their chemical composition, particularly their content of the anti-nutritional factors.

Conclusion

Halophytes for utilization are available in various regions and under different climates. The benefits of such use for the local freshwater saving are obvious, particularly in arid regions. Some applications of halophytes are highly profitable, for production of additional human food and for animal fodder. It is imperative that commercial production of halophytes should now be taken from research to large-scale field trials. Prospects of both short- and long-term production trials are well recognized and established. Halophytes could be a traditional source of animal nutrition even though they may present some problems.

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Biosaline Agriculture and Salinity Tolerance in Plants
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Potential of dry drainage as a sustainable solution to waterlogging and salinisation

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Introduction

It is estimated that, of the total 270 million hectares of irrigated land around the world, some 80 million hectares are affected by waterlogging and salinity, while about 20 million hectares suffer from severe irrigation-induced salinity problems [1].

Conventional wisdom holds that the best solution to dealing with salinity and waterlogging is to maintain a net flux of salt away from the rootzone and to control the water table by artificial drainage. A particular drainage may be suitable for local circumstances, but with large contiguous irrigation systems, there are two important general limitations, economic and environmental [2].

In recent years, there have been attempts to identify solutions which will work within environmental constraints and will also be economically viable [2, 3]. Improved on-farm water management combined with disposal by means of evaporation ponds is seen as the reasonable strategy, though with some environmental risks [3]. Another alternative is the control of the water level with irrigation management. A shallow water table can be considered as a valuable resource for meeting part of the crop requirement for water [4, 5]. However, in arid and semi-arid regions, the evaporative demand and the salinity of groundwater are usually high and the upward evaporative flux from a shallow saline water table results in the accumulation of salt to a very high concentration at or near the soil surface.

Salinity control depends upon establishment of a time averaged net downward flux through the rootzone. Therefore, it is the water balance that is important in determination of the balance. Within a given area, if inflow balances outflow, then the water table will be stable. If the non-cropped area is large enough and evaporation from this area is fast enough, then the necessary balance can be achieved without artificial drainage. This is the concept of dry-drainage. The groundwater system provides the pathway for the movement of the excess water from the irrigated land to the fallow land (Fig. 1).

The objectives of this study are i) to describe water and salt balances of both cropped and fallow areas, and ii) to assess the requirements for a successful dry-drainage system.

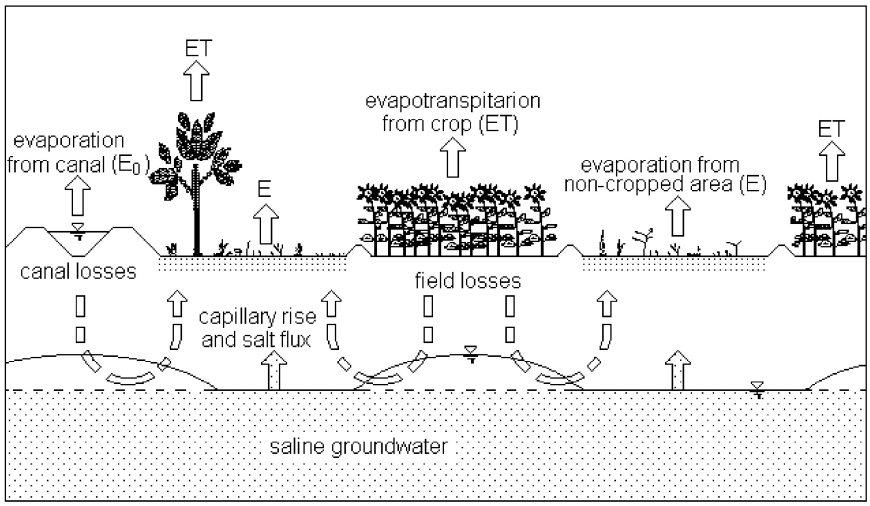

Figure 1. Schematic section of a dry-drainage system

Theory

As shown in Figure 1, a single dry-drainage unit has two parts, the irrigated area and the fallow area.

Design of the irrigated field

In arid and semi-arid areas, evapotranspiration (ET) exceeds the precipitation (P) and the resulting water deficit should be covered by irrigation (I) to achieve a satisfactory yield. The application of irrigation water means an input of salts because irrigation water, even if of excellent quality, is a major source of soluble salts. If soil salinisation is to be avoided, these salts have to be leached out by deliberate over-irrigation with the solution percolating to the subsoil. The leaching requirement (R^x) to provide the salt balance of the rootzone may be calculated following van Hoorn and van Alphen [6]. The total irrigation (I) then includes the leaching requirement as well.

The irrigation schedule is designed to maintain the salt balance during the whole year. Salt accumulation and leaching requirement of the irrigated area during the fallow period is computed as is in the fallow field.

Design of the fallow area

In investigating the sustainability of a dry-drainage system, we need to predict accurately the rate of evaporation and salt accumulation in the fallow area. Gowing et al. [7] developed a pseudo steady-state model, modifying the well-known Gardner [8] model, to predict the rate of evaporation from the soil surface, particularly from the surface of bare soil. Therefore, this model will be used to compute the rate

of evaporation from the fallow area. Accordingly they locate the depth of Evaporation Front (EF) and then calculate the rate of evaporation. The investigators have distinguished three stages in the progression of the EF: i) no EF exists, ii) the EF moves downwards, and iii) the EF is stationary [9]. The governing equations and calculation procedures for each of the stages are fully described in [7].

The concentration profile which develops with time depends upon both the upward evaporative flux of water, which concentrates salts, at the surface, and the diffusive–dispersive flux, which tends to move salts downward against the upward flux of water. Elrick et al. [10] model is applied to simulate salinity profile of the fallow area.

In the leaching process, the soil profile is considered as a series of one-dimensional reservoirs with bypass both in the fallow area after an effective rainfall and in the cropped area after a fallow period [6].

Parameters for simulating field behaviour

Average climatologic data and soil properties for the Lower Indus Basin in Pakistan are adopted from Asghar [2] (Tab. 1) and estimates of evapotranspiration (ET), are from Gowing and Wyseure [3] (Tab. 2). Salinity of the irrigation water, C_i, is 0.7 g/l [2, 3] whereas the salinity of the drainage water, C_s, is 2.8 g/l for a given crop pattern. The groundwater salinity of 7.0 g/l is taken in this study throughout the simulation period. A sandy clay loam soil prevails in the region [2]. The water table depth is taken as 1.5 m [2]. Monthly average precipitation (P) is distributed within a given month over an equal period taking the number of rains into consideration.

Table 1. Average climatologic data in the study area [2]. T, mean daily temperature; h_a, mean relative humidity of air; P, monthly mean precipitation and N, number of rains

Months	J	F	M	A	M	J	J	A	S	O	N	D
T(° C)	13	16.7	22.5	28.1	33.3	33.6	31.4	30.3	28.9	26.1	20.0	15.3
h_a (%)	57	51	36	27	28	45	67	72	62	44	41	56
P(mm)	23	18	13	8	13	74	180	173	117	10	3	10
N (d)	2	2	1	1	2	4	8	8	4	1	1	1

Table 2. Average evapotranspiration [3], irrigation and leaching requirements in the study area. ET_0, reference evapotranspiration; ET, evapotranspiration for wheat and cotton; I^x, irrigation without leaching ($I^x = ET - P$); I, total irrigation amount ($I = I^x + R^x$) and R^x, leaching requirement with 80 % leaching efficiency coefficient f_i+ field losses of 15 %)

Month	J	F	M	A	M	J	J	A	S	O	N	D
ET_0 (mm)	64	82	140	183	243	262	214	198	177	136	81	61
ET wheat (mm)	60	90	98								75	34
ET cotton (mm)				75	49	144	225	218	177	109		
I^x(mm)	59	96	113	89	48	93	60	60	80	132	96	32
R^x (mm)	10	17	20	16	8	16	11	11	14	23	17	16
I(mm)	69	113	133	105	56	109	71	71	94	155	113	48

Results and discussion

Water and salt balance of the cropped area

The amount of water (leaching + irrigation losses) percolating from the cropped area for each month during a year is given in Table 2. The maximum and minimum percolation occurred in October (27 mm) and December (7 mm), respectively.

Water and salt balance of the fallow area

Figure 2a shows the daily evaporation from the soil surface over a year: note that the calculation started from October but is presented from January. A relatively high evaporation rate on the first day (day 273), about 8 mm/d, decreased to 2.1 mm/d within the first 7 days because the evaporative demand of the atmosphere exceeded the ability of the soil to conduct water so causing the soil surface to dry. The evaporation rate then fluctuated minimally above this value during the dry season following small amounts of precipitation. Daily evaporation increased suddenly when the rainy season began and then fluctuated widely between the potential and limiting rates during the rainy season.

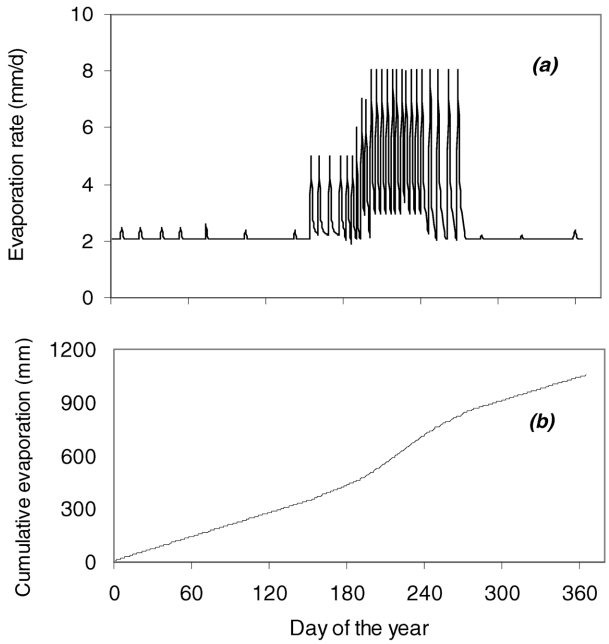

Figure 2. (a) Evaporation rate and **(b)** cumulative evaporation from the soil surface of the fallow area during a year

The cumulative evaporation from the fallow area should balance the total of precipitation and percolating water from the cropped area for dry-drainage to be a success. The cumulative evaporation from the fallow area was 1,054 mm/year (Fig. 2b) while the sum of precipitation (643 mm/year) and percolating water from the cropped area (198 mm/year) amounted to 841 mm/year. This means that the fallow field is capable of sustaining the required water balance for the success of the system. Under the simulated conditions, the cropped area may be larger than the fallow area by a factor of 1.25 (i.e., 1,054/841).

The salt-concentration profile was calculated monthly. The salt and water profiles at the end of the previous month were used as the initial conditions for the next month.

Figure 3 shows the calculated salt concentration profiles at four different times during the dry period. At the end of the dry season, approximately the top 60 cm of soil layer had become saline. Salt concentration in the top 10 and 20 cm did not change after the end of January and May because of the formation of an evaporation front in which water was considered to move as vapour. Leaching was calculated during the rainy season.

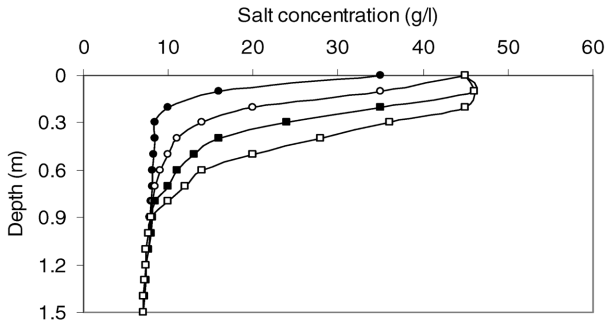

Figure 3. Profiles of salt concentration of the fallow area at four different times during the dry period. ●: 30 November, ○: 31 January, ■: 31 March, □: 31 May

After each rain, the water and salinity profiles were recalculated. Figure 4 shows the calculated salt concentration of each soil layer. The amount of evaporation during the period between two rainfalls was allowed for calculating the next water-content profile. Although the rainy season started from June, the amount of precipitation during this month was not sufficient to replenish the water content to field capacity so no percolation and therefore no leaching occurred. At the end of July, a considerable amount of salt from the 0–30 cm soil layer was leached into the 30–60 cm layer but there was no leaching below the 30–60 cm layer. During August and September, leaching occurred in all soil layers; however, a small amount of salt still remained in the soil at the end of the year.

Having carefully considered the water and salt balance of both irrigated and fallow area, the remaining salt in the soil profile of the fallow area at the end of the year may be leached if this leaching requirement is not too large, as practiced

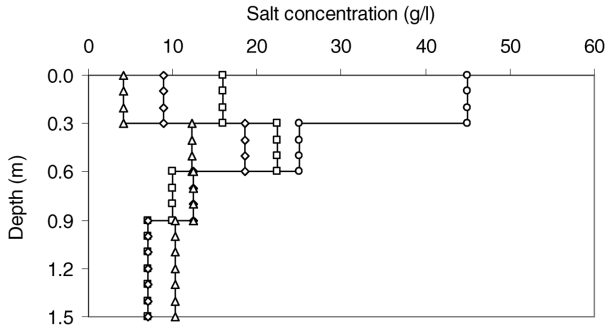

Figure 4. Changes in the salt concentration of the soil layers leached after four different times during the rainy period. ○: initial, □: 30 July, ◇: 31 August, △: 30 September

in West Africa [2]. In our case, 120 mm water is needed. Re-checking the water balance of the fallow land, the amount of inflow (961 mm) needed, which is the sum of percolating water from the irrigated area (198 mm), total precipitation (643 mm) and leaching requirement of fallow field (120 mm), is still smaller than that of the outflow which is the cumulative evaporation from the fallow field, 1,054 mm. In this case, the irrigated area may be approximately 10 % larger than the fallow area, which is virtually the same as proposed by [2, 3].

Conclusions

The rate of evaporation the fallow area was fast enough to cover the net downward flux through the cropped area due to the leaching requirement and 15 % irrigation losses when the groundwater was stable at 1.5 m depth. Under the simulated conditions, the necessary water balance could be achieved when the cropped area was larger than the fallow area by a factor of 1.25. However, the salt balance of the fallow area was not satisfied. If this salt is to be leached at the end of the growing season, the ratio of cropped to fallow areas becomes 1:10. However, this ratio may change with climate, soil type, water table depth, irrigation amount and groundwater quality and crop pattern.

About 50 % of the potentially irrigable land should be abandoned, which may be hard to accept. But, given the choice of paying the full economic cost of a regional drainage scheme, would farmers find it so unattractive? A full economic analysis is needed.

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Vegetative bioremediation of sodic and saline-sodic soils for productivity enhancement and environment conservation

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Introduction

Salt-affected soils occupy nearly 20 % of irrigated area worldwide [1]. As a major category of salt-affected soils, sodic and saline-sodic soils are characterized by the occurrence of sodium (Na^+) at levels that result in poor physical properties and fertility problems, thereby threatening agricultural productivity in many arid and semi-arid regions. Amelioration of these soils is driven by providing a soluble source of calcium (Ca^{2+}) to replace excess Na^+ on the cation exchange complex [2]. The displaced Na^+ is either leached from the root zone by excess irrigation, a process that requires soil permeability and provision of a natural or artificial drainage system, or is taken up by crops.

Many sodic and saline-sodic soils contain inherent or precipitated sources of Ca^{2+}, i.e., calcite ($CaCO_3$) at varying depths. Due to its negligible solubility (0.14 mmol L^{-1}), natural dissolution of calcite does not provide sufficient Ca^{2+} to ameliorate these soils. Consequently, amelioration of these soils has been dominated by the application of chemical amendments [3]. Some amendments supply soluble sources of Ca^{2+} to the soil solution, which then replace excess Na^+ on the exchange complex, while others assist in increasing the dissolution rate of calcite. There have been constraints with chemical amelioration in several developing countries during the last two decades because of 1) low-quality of amendments containing a large fraction of impurities, 2) restricted availability of amendments when needed for amelioration, and/or 3) increased costs of amendments due to competing demands by industry and reductions in government subsidies for their agricultural use. Owing to overriding importance of the last factor, chemical amelioration has become prohibitively expensive for resource-poor farmers. However, there is growing evidence from researchers and farmers indicating that these soils can be brought back to a highly productive state

using a plant-assisted amelioration approach – vegetative bioremediation – that does not rely on chemical amendments [4–6]. Synonymous terms for vegetative bioremediation include phytomelioration, phytoremediation, and biological reclamation.

Typical plant-assisted amelioration strategies for contaminated soils, such as those containing elevated levels of heavy metals and metalloids, work through cultivation of specific plant species capable of hyper-accumulation of target ionic species in their shoots, thereby removing them from the soil [7]. In contrast, vegetative bioremediation of sodic and saline-sodic soils is achieved by the ability of plant roots to increase the dissolution rate of calcite, thereby resulting in enhanced levels of Ca^{2+} in soil solution. The salinity-sodicity combination present in the soil solution during vegetative bioremediation maintains adequate soil structure and aggregate stability that enhance the amelioration process [8]. This chapter highlights the role of cropping for vegetative bioremediation of calcareous sodic and saline-sodic soils and its evaluation against other amelioration approaches. This information will assist researchers and farm advisors in choosing appropriate crops as well as crop, soil and irrigation management practices to achieve maximum benefit during the amelioration process.

Vegetative bioremediation of sodic and saline-sodic soils

Vegetative bioremediation of calcareous sodic and saline-sodic soils is a promising option that increases the dissolution rate of calcite through the processes at the soil-root interface resulting in enhanced levels of Ca^{2+} in soil solution. Vegetative bioremediation (V_{Bio}) is a function of the following factors:

$$V_{\mathrm{Bio}} = \sum R_{\mathrm{PCO2}} + R_{\mathrm{H^+}} + R_{\mathrm{Phy}} + S_{\mathrm{Na^+}} \tag{1}$$

where $R_{\mathrm{P_{CO_2}}}$ refers to increased partial pressure of CO_2 within the root zone, $R_{\mathrm{H+}}$ is enhanced proton (H^+) release in the root zone in case of certain N_2-fixing crops, R_{Phy} deals with physical effects of roots in improving soil aggregation and hydraulic properties of the root zone, and $S_{\mathrm{Na^+}}$ consists of Na^+ content of shoot which is removed through harvest of aerial plant portion. The collective effects of these factors ultimately lead to soil amelioration, provided leaching and drainage are adequate (Fig. 1).

Comparative efficiency of vegetative bioremediation

The evaluation of vegetative bioremediation and chemical approaches in various countries reveals comparable performance of both in terms of sodic soil amelioration. Results of a field experiment conducted on a barren, calcareous, alkali soil ($pH_{1:2}$ = 10.6, $EC_{1:2}$ = 2.7 dS m^{-1}, ESP = 94) indicated that the amelioration efficiency of two grasses, Para grass (*Brachiaria mutica* (Forssk.) Stapf) and Karnal grass (*Leptochloa fusca* (L.) Kunth), was comparable with soil application of gypsum at 12.5 Mg ha^{-1} [9]. The yield of first rice (*Oryza sativa* L.) crop in the gypsum treatment averaged 3.7 Mg ha^{-1} as compared to 3.8 and 4.1 Mg ha^{-1} from the treatments cropped for 1 year with Para and Karnal grasses, respectively. The corresponding

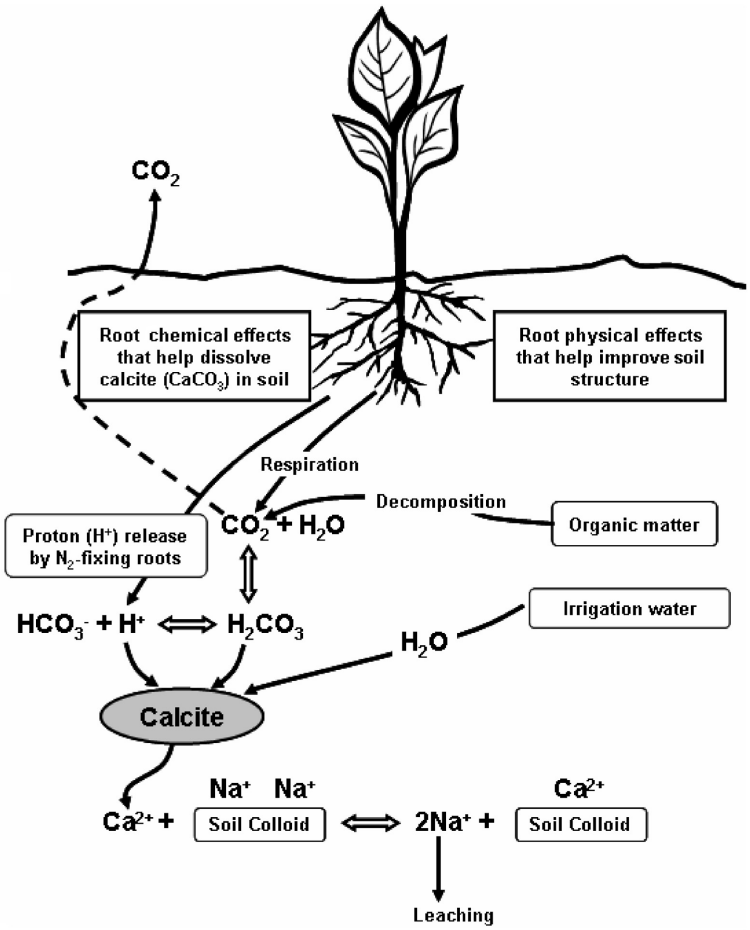

Figure 1. A conceptual model for the chemical reactions involved in calcite ($CaCO_3$) dissolution and amelioration of calcareous sodic and saline-sodic soils during vegetative bioremediation

rice yields after 2 years of grass cropping were 5.3 and 6.1 Mg ha^{-1}. In another field experiment [10], amelioration efficiency of Kallar grass was evaluated during different periods of root decay by leaching a calcareous, silty clay loam, saline-sodic soil (pH_s = 8.3–9.3, EC_e = 16.8–37.5 dS m^{-1}, SAR = 32.5–108.9) 3, 6, 9, and 12 days after each harvest during 2 years of grass cultivation. Each plot was kept flooded for 3 days during leaching. The amelioration efficiency of Kallar grass was greater in the plots leached 6 days after harvesting, and it was comparable with gypsum-treated soil.

In a field study [11], cropping of sesbania (*Sesbania bispinosa* (Linn.) W.F. Wight), Kallar grass, and sordan (*Sorghum* ×*drummondii* (Steud.) Millsp. & Chase) was compared against gypsum application (13 Mg ha^{-1}) on a calcareous, sandy clay

loam, saline-sodic soil (pH_s = 8.2–8.6, EC_e = 7.4–9.0 dS m^{-1}, SAR = 55.6–73.0). The plant species were grown for two seasons (15 months) with average forage yields in the order: sesbania (40.8 Mg ha^{-1}) > Kallar grass (29.3 Mg ha^{-1}) > sordan (24.7 Mg ha^{-1}). After two cropping seasons, the treatment efficiency for grain yield of the subsequent wheat (*Triticum aestivum* L.) crop was in the order: sesbania (3.79 Mg ha^{-1}) $\approx$ gypsum (3.68 Mg ha^{-1}) > Kallar grass (3.14 Mg ha^{-1}) > sordan (2.27 Mg ha^{-1}) > control (0.65 Mg ha^{-1}). In a later field experiment [5], four plant species – Kallar grass, sesbania, millet rice, and finger millet – were tested against gypsum application (14.8 Mg ha^{-1}) to ameliorate a calcareous, sandy clay loam, saline-sodic soil (EC_e = 9.1–11.0 dS m^{-1}, SAR = 59.4–72.4). The treatment effectiveness to decrease soil EC_e and SAR was in the order: gypsum $\approx$ sesbania > Kallar grass > millet rice > finger millet. Forage yields of the plant species were directly proportional to their soil amelioration efficiency.

Some field trials of crop bioremediation techniques have not been successful primarily because a salt-resistant forage crop was not the first crop in the rotation. In a field experiment [12], biological (rice-wheat rotation), physical + biological (subsoiling by curved chisels to a depth of 0.5 ± 0.05 m at a chisel spacing of 1.2–1.5 m + rotation), chemical + biological (gypsum at 100 % gypsum requirement of the upper 0.15 m of soil + rotation), and chemical + physical + biological (gypsum + subsoiling + rotation) methods were compared to ameliorate two calcareous saline-sodic soils. Irrigation water (EC = 1.8 dS m^{-1}, SAR = 9.8) was applied according to the crop water requirement. The first crop in the rotation was rice, which was a complete failure and did not produce any grain on one soil (pH_s = 8.6–9.1, EC_e = 12.3–15.0 dS m^{-1}, ESP = 58.7–74.6), and a grain yield of 0.72 Mg ha^{-1} on the other soil (pH_s = 8.8–8.9, EC_e = 9.6–15.2 dS m^{-1}, ESP = 42.5–45.6). Four years after cropping, the average rice grain yield from both soils was in the order: gypsum (1.99 Mg ha^{-1}) > gypsum + subsoiling (1.84 Mg ha^{-1}) > subsoiling (1.41 Mg ha^{-1}) > vegetative bioremediation (1.02 Mg ha^{-1}). Gypsum and gypsum + subsoiling treatments had similar values for the wheat grain yield (2.72 Mg ha^{-1}) followed by subsoiling (1.79 Mg ha^{-1}) and vegetative bioremediation (1.46 Mg ha^{-1}). Within the upper 0.15 m depth, all the treatments decreased EC_e levels less than 5 dS m^{-1} and ESP levels less than 22 on both the soils.

Several crop rotations have been evaluated for the amelioration of saline-sodic and sodic soils. Three irrigated crop rotations were tested to ameliorate a calcareous saline-sodic field (pH_s = 8.1–8.2, EC_e = 9.2–13.7 dS m^{-1}, SAR = 30.6–42.7). The rotations distributed in plots of 18 m^2 were: sesbania-barley (*Hordeum vulgare* L.), rice-wheat, and Kallar grass-alfalfa (*Medicago sativa* L.). All the crop rotations reclaimed the upper 0.15 m of soil after 1 year (SAR < 10) as did amelioration by the non-cropped gypsum treatment, which decreased SAR less than 14 [13]. Although initial salinity and sodicity levels of this soil were closer to that used by [12], there were three differences: 1) the soil was relatively coarser in texture, 2) the plots were irrigated with canal water (EC = 0.3 dS m^{-1}, SAR = 0.5), and 3) the irrigation water was applied in excess of crop water needs to leach Na^+ to lower depths.

In an evaluation of 14 experiments, carried out in different parts of the world, there was a comparable effect of chemical and bioremediation approaches [14]. The chemical treatment (application of gypsum in all experiments) caused 62 % decrease

in original sodicity levels, whereas a 52 % decrease was calculated for the vegetative bioremediation treatments. However, in some experiments bioremediation was either unsuccessful or much less efficient than the chemical treatment for the reasons: 1) a crop resistant to ambient soil salinity and sodicity levels was not the first in the crop rotation; 2) bioremediation crop was grown during the time, which was not its most suitable growing season; 3) time was insufficient to exploit the potential impact of the bioremediation crop; and/or 4) irrigation was not applied in excess of crop water requirement, which restricted the downward movement of Na^+ from the root zone. In general, bioremediation worked well on moderately sodic and saline-sodic soils, provided: 1) irrigation was in excess of crop water requirement to provide adequate leaching; and 2) the excess irrigation was applied when the crop growth and hence P_{CO_2} were at their peak. On these soils, the performance of vegetative bioremediation was comparable with soil application of gypsum. On highly sodic and saline-sodic soils, chemical treatment was better than the cropped treatments.

Additional benefits of vegetative bioremediation

Nutrient availability status of post-amelioration soil is crucial for the growth of subsequent crops. Research on nutrient behavior during amelioration using chemical and biological methods has been conducted by determining the availability status of some macro- and micro-nutrients during amelioration of a calcareous saline-sodic soil (pH_s = 8.2–8.6, EC_e = 7.4–9.0 dS m^{-1}, SAR = 55.6–73.0). The bioremediation treatments included cropping of sesbania, sordan, or Kallar grass for 15 months. There was an increase in phosphorus (P), zinc (Zn), and copper (Cu) availability in the bioremediation plots resulting from the production of root exudates and likely dissolution of some nutrient-coated calcite. Conversely, the non-cropped gypsum treatment decreased the availability status of these nutrients. Besides leaching losses, adsorption of nutrients on some newly formed $CaCO_3$, a secondary consequence of gypsum dissolution, contributed to this decrease. Soil N content was decreased in all the treatments except for N_2-fixing sesbania treatment where N content was increased from 0.49 g kg^{-1} to 0.53 g kg^{-1}. There was no treatment effect on soil potassium (K) availability since illite, a K-bearing mineral, was dominant in the clay fraction [15].

Soil microbial biomass is an agent of transformation for added and native organic matter and acts as a labile reservoir for several plant-available nutrients. The activity of microbial biomass is commonly used to characterize microbiological status of a soil and to determine the effects of agricultural practices on soil microorganisms. Dehydrogenase activity (DHA) in soils is related to microbial populations, respiration activity and soil organic matter, and provides an index of the overall microbial activity [16]. This parameter has been studied in few experiments dealing with sodic soil amelioration through chemical and biological means. After using several combinations of chemical and vegetative bioremediation treatments, DHA and microbial biomass carbon (MBC) were determined [17]. The treatments consisted of Karnal grass grown for 1 or 2 years (harvested biomass removed or left to decompose on the soil surface), gypsum application (at 14 Mg ha^{-1}) + Karnal grass, gypsum + sorghum, gypsum + rice, and gypsum + sesbania. The soil on which these treatments were applied was alkali ($pH_{1:2}$ = 10.6, $EC_{1:2}$ = 2.1 dS m^{-1}, ESP = 95, DHA

= 4.5 μg triphenylformazan g^{-1}, MBC = 56.7 mg kg^{-1}). The levels of DHA in post-amelioration soil were greater (118.7 μg triphenylformazan g^{-1}) in the bioremediation treatments than gypsum + crop treatments (96.1 μg triphenylformazan g^{-1}). The MBC values were greater in gypsum + crop treatments (206.3 mg kg^{-1} soil) than in the cropped treatments (161.7 mg kg^{-1} soil). The overall experimental average of MBC (184 mg kg^{-1} soil) for all the treatments was more than three times the initial level of 56.7 mg kg^{-1} soil. In an earlier study [18], a significant increase in urease and dehydrogenase activities was found in alkali soils under permanent vegetation such as grasses. Green manuring of an alkali soil with sesbania has also been reported to increase urease and dehydrogenase activities [19].

Sodic and saline-sodic soils have lost a large fraction of their original carbon (C) pool [20]. The magnitude of the loss may range between 10–30 Mg C ha^{-1}, depending on the antecedent pool and the severity of degradation. The soil C pool is not only important for the soil to perform its productivity and environmental functions, but also plays an important role in the global C cycle. In addition to amelioration effect, cultivation of appropriate crops, shrubs, and trees on sodic and saline-sodic soils has the potential to mitigate accelerated greenhouse effects by increasing soil C through biomass production (Tab. 1). Monitoring changes in an alkali soil cropped with four tree species – acacia (*Acacia nilotica* Willd ex Delile), shisham (*Dalbergia sissoo* Roxb. ex DC.), mesquite (*Prosopis juliflora* (Swartz) DC.) and arjuna (*Terminalia arjuna* Bedd.) – suggested shisham and mesquite as more efficient in terms of biomass production and decreasing Na^+ levels in the soil. Similarly, there was greater microbial activity in upper 0.6 m soil under these species due to the accumulation of humus from decomposition of leaf litter and root decay, which increased soil organic C. The rate of increase was low for the first 2–4 years, exponential between 4–6 years, and plateau at a low rate for 6–8 years [21]. Establishment of mesquite on a sodic field increased organic C of the top 1.2 m soil from 11.8 Mg C ha^{-1} to 13.3 Mg C ha^{-1} in 5 years, 34.2 Mg C ha^{-1} in 7 years, and 54.3 Mg C ha^{-1} in 30 years. The average annual rate of increase in soil organic C was 1.4 Mg ha^{-1} over the 30-year period [22]. Other estimates from field studies on alkali soils suggest that different land-use systems consisting of a number of grasses and trees can sequester organic C in the range of 0.2–0.8 Mg C ha^{-1} yr^{-1} [6].

Plant species for vegetative bioremediation

The selection of plant species for vegetative bioremediation is generally based on the ability of the species to withstand ambient levels of soil salinity and sodicity while also providing a saleable product or one that can be used on-farm. Considerable variation exists among crops to withstand saline-sodic conditions [23]. Such inter- and intra-crop diversity suggests that field trials be conducted to identify local crops that are adaptable to saline-sodic soil conditions [24]. The farmers, farm advisors, and researchers familiar with local conditions, including crop response to adverse soil conditions and cropping strategies that fit into the local economic conditions, could provide a valuable resource base for making appropriate recommendations. In addition, application of plant breeding approaches is needed to develop crop genotypes with enhanced salt resistance and performance in field conditions [25].

Table 1. Potential of two land-use systems (grass only and tree-grass) for carbon (C) sequestration in a calcareous alkali soil (pH = 10.0–10.2; EC = 2.0–6.4 dS m^{-1}). Recalculated from [6]

Treatment[a]	Organic C in soil (g kg^{-1}) at different depths[b]			C sequestration (Mg ha^{-1} yr^{-1})[c]
	0–0.075 m	0.075–0.15 m	Mean	
Desmostachya	2.9	1.6	2.3	0.33
Sporobolus	2.4	1.3	1.8	0.17
Acacia + *Desmostachya*	3.6	1.8	2.7	0.47
Dalbergia + *Desmostachya*	4.6	2.4	3.5	0.73
Prosopis + *Desmostachya*	4.7	2.5	3.6	0.77
Acacia + *Desmostachya*	2.6	1.4	2.0	0.23
Dalbergia + *Desmostachya*	3.2	1.7	2.5	0.40
Prosopis + *Desmostachya*	3.6	1.9	2.8	0.50

[a] *Desmostachya* (*Desmostachya bipinnata* (L.) Stapf), *Sporobolus* (*Sporobolus marginatus* Hochst. ex A. Rich), *Acacia* (*Acacia nilotica* (L.) Delile), *Dalbergia* (*Dalbergia sissoo* Roxb. ex DC), *Prosopis* (*Prosopis juliflora* (Sw.) DC)

[b] After 6 years of plantation

[c] Assuming initial C content in the soil as 1.3 g kg^{-1} (average of the C content, which ranged from 1.0–1.6 g kg^{-1}) and mass of 0.15 m depth of 1 ha as 2×10^6 kg, the rate of organic C sequestration in the soil under each treatment was calculated as:
Organic C sequestr. (Mg ha^{-1} yr^{-1}) = [(mean C content – original C content in soil) 2] / 6

Several crops, shrubs, trees, and grasses have been used as vegetative bioremediation tools to ameliorate sodic and saline-sodic soils. Some researchers have favored the inclusion of Kallar grass [9], sesbania [11], alfalfa [26], Bermuda grass [8], or sordan [4] as the first crop to accelerate sodic soil amelioration. Several other plant species have produced adequate biomass on salt-affected soils. These include shrub species from the genera *Atriplex* and *Maireana* [27, 28], *Kochia scoparia* L. [29], *Salicornia bigelovii* Torr. [30], *Echinochloa crusgalli* (L.) P. Beauv. [31], and *Portulaca oleracea* L. [32], among others. However, it is imperative to compare them with other species already tested for sodic soil amelioration. In addition, efforts are needed to search other crops such as high-value medicinal and aromatic species with the potential for adequate growth on sodic and saline-sodic soils.

A number of tree plantations have been grown on sodic and saline-sodic soils. These include: *Terminalia arjuna* (Roxb. ex DC.) Wight and Arn. [33], *Prosopis juliflora* (Sw.) DC. [22], *Dalbergia sissoo* Roxb. ex DC., *Acacia nilotica* (L.) Willd. ex Delile [6], *Parkinsonia aculeata* L. and *Prosopis cineraria* (L.) Druce [34], *Sesbania sesban* (L.) Merr. and *Tamarix dioica* Roxb. ex Roth [35], and *Leucaena leucocephala* (Lam.) de Wit [36], among others. In Australia, revegetation by trees was found to be the best long-term option for controlling dryland salinity [37]. Useful information is available regarding sources of seeds, nursery raising techniques, and land preparation

and planting procedures for 18 different tree and shrub species with potential for growth on salt-affected soils [34].

Based on cost and benefit analysis, several studies have compared economics of sodic soil amelioration. A net economic loss (cost:benefit 1.00:0.75) was found during vegetative bioremediation although the growth of Karnal grass was adequate, which helped reduce soil sodicity. The economic loss was attributed to the small market demand of the grass in the presence of other good-quality forages in that locality [38]. On the other hand, the bioremediation strategy has been found to be economically beneficial when there was a market demand or local utilization of the crops at the farm level [39, 40]. Agroforestry systems comprising several tree species on saline-sodic soils have been found to be economically feasible in some developing countries because of firewood need in local markets [36]. On the other hand, the market for firewood is not supportive to make agroforestry economically viable in California [8]. Preliminary assessments in Australia suggest that there are 26 salt-resistant plant species capable of producing 13 products (or services) of value to agriculture [27]. From an economic perspective much depends on local needs. In an immediate sense, vegetative bioremediation can only be economically feasible if the selected crops, grasses, or trees have a market demand or local utilization at the farm level. In the long run, one must also consider the value of the improved soils.

Conclusions

In recent decades, vegetative bioremediation has been found to be an efficient, inexpensive, and environmentally acceptable strategy to ameliorate sodic and saline-sodic soils. Its comparable performance with that of chemical amelioration highlights the effective role of cropping in the amelioration of these soils. Vegetative bioremediation has shown to be advantageous in several aspects: 1) no financial outlay to purchase chemical amendments, 2) accrued financial or other benefits from crops grown during amelioration, 3) promotion of soil-aggregate stability and creation of macro-pores that improve soil hydraulic properties and root proliferation, 4) greater plant-nutrient availability in soil after vegetative bioremediation, 5) more uniform and greater zone of amelioration in terms of soil depth, 6) sequestration of C in post-amelioration soil, and 7) environmentally feasible and productive use of otherwise marginal and degraded soils. However, vegetative bioremediation is slower in effecting positive change than chemical approaches and is contingent on the presence of calcite in soil, which is common when compared to most sodic and saline-sodic soils of arid regions. In addition, its scope becomes limited on highly sodic soils where growth of the bioremediation crops is likely to be variable and patchy and the use of chemical amendments such as gypsum is inevitable. Clearly, vegetative bioremediation is an effective low-cost intervention for resource-poor farmers. This approach has the potential for large-scale adoption under government or community-based programs aimed at the amelioration and improved productivity of degraded common property resources.

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Biosaline Agriculture and Salinity Tolerance in Plants
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Bio-reclamation of secondary salinized soils using halophytes

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Soil salinity has become one of the major determinants of global crop productivity. Consequently reclamation of such soils is a most urgent requirement for world food production and for sustainable development. Out of an estimated area of 173 million hectares of totally degraded land in India, approximately 7 million hectares are affected by salinity. Besides naturally occurring saline soils, the secondary salinity that developed due to saline water irrigation is posing a great threat to the perspectives of increasing food and fodder production. In arid and semi-arid regions like Rajasthan (India) decreasing water table and increased use of deep wells, following electrification of villages, has resulted in an increased salinity of irrigation water and consequently in increased salinization. Although, in context with the increasing population, the importance of irrigated agriculture cannot be ignored and excessive saline water irrigation may convert productive soil into unproductive and salinized soil. In western Rajasthan, the area that is affected by the use of such problematic water is some 880 km^2. The irrigation water being used there is moderate to highly saline/sodic [1, 2]. As such a large percentage of land is going out for production, year by year, due to saline water irrigation it is a major contributory factor to soil degradation in India [3]. Several halophytic plant species have been tried in the past for their possible use in reclamation of salt-affected soils [4–8]. Besides their positive impact on salt-affected soils, the potential use of some halophytes as forage and as oil seed crops has also been described [9]. However, use of halophytes for soil reclamation is still in an exploratory stage and only a few field studies for bio-reclamation of saline soil using halophytes have been carried out so far [5, 10]. Therefore keeping this fact in mind, the present investigation was undertaken with the objective to study the utilization of halophytes to remove excess salinity added by irrigation.

Materials and methods

Plant species

Salsola baryosma (Roem and Schult), *Haloxylon recurvum* (Moq.) Bunge ex. Boiss, *Suaeda nudiflora* (Willd.) Moq. (all Chenopodiaceae) were studied in the present investigation. Plants were identified according to Bhandari [11] and dried specimens were deposited in the herbarium of the Department of Botany, University of Rajasthan, Jaipur.

Experimental site

Experiments were conducted at Pachkodia village (district of Jaipur), located at the latitude 26° 5'N, longitude 75° 28'E and altitude of 427 m. This area represents the soil and agro-climatic conditions of about two thirds of Rajasthan. The climate of this area is semi-arid with an average rainfall of approximately 500 mm, with more than 80 % of its precipitation during the months of July and August. Temperatures fluctuate widely during the year, ranging from as high as 45°C in summer to 2–4°C in winter.

Experimental plots

14 field plots of 13.5 m^2 each were prepared for each treatment. Sowing was carried out by mixing the seeds in the upper 3 cm layer of the soil. A 5 m space was kept between treatments to ensure that there is no seeping of any mineral or water from one treatment to another.

Treatments

T_1-field plots planted with *Salsola baryosma.*
T_2-field plots planted with *Haloxylon recurvum*.
T_3-field plots planted with *Suaeda nudiflora*.

Irrigation of the plants

Irrigation was carried out two times over a period of 3 months. The irrigation comprised of approximately 10–15 cm depth of water in all the plots. The flood irrigation method was used for irrigation, which is of usual practice in the area. Irrigation water used was of the C_4-S_4 category [12], having high sodium absorption ratio (SAR), high pH value and high electric conductivity (Tab. 1).

Soil samples

Initial soil samples were collected prior to plantation and referred to as 'initial' soil samples. Seed sowing was carried out. Plants were allowed to grow for 3 months and

Table 1. Composition of irrigation water used for irrigation at experimental site

Determination	Value
pH	8.04
EC	8750.00 $\mu S.cm^{-1}$
K^+	4.80 $mg.L^{-1}$
Na^+	1845.00 $mg.L^{-1}$
Mg^{2+}	29.70 $mg.L^{-1}$
Ca^{2+}	83.20 $mg.L^{-1}$
SAR*	39.30
F^-	65.41 $mg.L^{-1}$
Cl^-	1010.70 $mg.L^{-1}$
$NO_3{}^{2-}$	5.48 $mg.L^{-1}$
$SO_4{}^{2-}$	1222.30 $mg.L^{-1}$

*SAR was calculated following [12]

then soil samples were collected and referred to as 'final' soil samples. Soil samples were taken from every plot and were collected from five different depths, i.e., 0–10, 10–20, 20–30, 30–40 and 40–50 cm.

Climatic condition during the experimental period

During the study period the maximum temperature was in the range of 25–41.7° C. Maximum humidity during the experimental period ranged from 52% to almost 100% on some rainy days. Rainfall during the experimental period ranged between 0.4 mm to 60.4 mm.

Plant analysis

The total aerial shoot was cut at the soil line and then dried at 105° C until the weight became constant. Fresh weight and dry weight were determined. Subsequently, dried aerial shoot was ground in a coffee mill up to 1 mm size. 100 mg plant material was placed in porcelain crucibles and ashing was carried out for approximately 20 h at 550° C in a muffle furnace until the organic matter completely disappeared. Ashes were then digested in 50 % v/v nitric acid and diluted with distilled water for analysis. Potassium and sodium were determined using flame emission and Mg^{2+} and Ca^{2+} were determined using atomic absorption.

Soil and water analysis

The pH and EC were determined in 1:2.5 (soil:water) extract where extracts were prepared without using vacuum or pressure. For determination of cations, extracts were prepared following Mehlich [13], while using barium chloride as exchanger. Exchangeable sodium, exchangeable potassium, exchangeable calcium and exchange-

able magnesium were determined using atomic absorption spectrophotometry (Perkin Elmer model 2380). Exchangeable sodium percentage was calculated following Richards [12].

Results

Plant analysis

Table 2 presents the ion composition and biomass production in plants undertaken in the present investigation.

Table 2. Ion accumulation, Na^+ uptake and biomass production in *S. baryosma*, *S. nudiflora* and *H. recurvum* over a period of 3 months

Plant species	Ion accumulated mg.g^{-1}.dry wt.				Sodium uptake g.plant^{-1}	Shoot biomass produced kg.dry wt.ha^{-1}
	K^+	Na^+	Mg^{2+}	Ca^{2+}		
S. baryosma	3.44	68.16	5.59	9.21	9.61	1,847
S. nudiflora	5.94	89.86	5.09	6.16	15.63	2,175
H. recurvum	5.94	67.59	5.60	5.34	17.03	2,192

Effect of halophyte plantation on soil characteristics

Soil reaction (pH)

The pH of the soil was considerably modulated by halophyte plantation (Tab. 3). In *S. baryosma* plots a considerable decrease in soil pH was observed in all the depth levels. However in *S. nudiflora* plots, an increase in soil pH was recorded in 10–20 and 20–30 cm soil layers. However soil pH was decreased in 0–10 and 30–50 cm soil layers. In *H. recurvum* plots, soil pH decreased considerably, in all the depth levels. In control plots an increase in pH was recorded in all depth levels.

Soil salt content (EC)

Soil salt content markedly differed in initial and final soil samples as indicated by soil electric conductivity (Tab. 3). In *S. baryosma* planted plots a considerable decrease in soil EC was observed in 10–40 cm soil layers. However an increase in soil EC was recorded in 0–10 and 40–50 cm soil layers. In *S. baryosma* plots maximum reduction in soil EC was recorded in 10–20 cm soil layer, which was followed by 20–30 cm and 30–40 cm soil layers. In *H. recurvum* grown field plots, soil electric conductivity (EC) decreased in all depth levels. The reduction in soil EC was 56% to 85% in different depth levels. Maximum reduction (85%) in EC was recorded in 10–20 cm and minimum reduction (56%) was in the 40–50 cm depth layer. In *S.*

Table 3. Effect of halophyte plantation on some physical and chemical characters of secondary salinized soil. Final values with different letter (a, b, c) differs significantly from initial values ($P < 0.05$) according to T-test

		S. baryosma plots		*S. nudiflora* plots		*H. recurvum* plots		Control plots	
Determination	Depth	Initial	Final	Initial	Final	Initial	Final	Initial	Final
pH	0–10	8.16 a	8.13 a	8.13 a	8.04 b	8.44 a	7.73c	8.10 a	8.42 b
	10–20	8.29 a	8.03 b	7.99 a	8.04 a	8.27 a	7.96c	8.08 a	8.33 b
	20–30	8.48 a	7.88 c	7.97 a	8.02 a	8.27 a	8.01c	7.90 a	8.16 b
	30–40	8.32 a	7.90 c	7.99 a	7.97 a	8.38 a	7.95c	7.95 a	7.96 a
	40–50	8.27 a	7.65 c	7.93 a	7.82 b	8.01 a	7.74c	7.88 a	7.89 a
EC $_{(1:2.5)}$ ($\mu S.cm^{-1}$)	0–10	768 a	967 b	930 a	199 c	1155 a	314 c	868a	852.5 b
	10–20	1068 a	697 c	1142 a	167 c	1880 a	273 c	665 a	710. b
	20–30	1002 a	707 c	1092 a	167 c	1006 a	228 c	448 a	639.5 b
	30–40	971 a	754 c	949 a	175 c	939 a	246 c	421 a	629.5 b
	40–50	858 a	1200 b	585 a	233 c	648 a	279 c	421 a	581.0 b
Na^+ ($mg.100g^{-1}$)	0–10	33.75 a	97.25 c	84.75 a	47.75 b	74.25 a	33.75 c	64.50 a	104.25 b
	10–20	64.50 a	90.75 b	45.00 a	63.75 b	80.00 a	37.75 b	74.50 a	98.25 b
	20–30	69.75 a	89.25 b	75.25 a	76.25 a	81.25 a	35.00 b	79.00 a	98.25 b
	30–40	73.50 a	96.25 b	78.50 a	81.25 a	77.23 a	39.00 c	80.00 a	92.00 b
	40–50	66.00 a	106.50 b	70.25 a	92.25 b	64.00 a	42.75 b	63.50 a	99.25 b
Ca^{2+} ($mg.100g^{-1}$)	0–10	12.00 a	17.00 c	7.75 a	22.25 b	5.50 a	15.75 c	19.50 a	11.50 b
	10–20	13.25 a	6.50 b	11.00 a	19.50 b	8.50 a	19.00 b	16.50 a	10.50 b
	20–30	14.25 a	8.00 b	9.75 a	18.00 b	10.25 a	14.50 b	11.75 a	9.75b
	30–40	15.75 a	7.00 b	13.00 a	19.00 b	12.00 a	10.50 c	12.75 a	8.75b
	40–50	14.00 a	12.00 a	11.00 a	18.00 b	11.75 a	13.50 b	13.25 a	10.00b
ESP	0–10	50.58	77.95	81.8	55.31	80.79	46.01	62.3	79.0
	10–20	70.35	87.18	75.8	64.57	79.47	44.63	60.5	80.6
	20–30	73.09	84.59	74.5	70.84	77.99	46.44	66.1	80.09
	30–40	73.86	86.30	75.6	70.35	74.79	51.73	76.6	80.20
	40–50	72.70	82.64	63.5	74.13	71.56	50.61	66.1	76.07

nudiflora plots a significant decrease in soil EC was also recorded at all depth levels. Reduction in soil EC ranged from 60% to 85% in different depth levels. Maximum reduction (85%) in soil EC was recorded for 10–20 cm soil layer, which was followed by 20–30 cm soil layer where 84% reduction for soil EC was recorded. In *S. nudiflora* grown field plots, least reduction in soil EC was recorded in 40–50 cm soil layer. In control plots, where no halophytes were grown, an increase in soil EC was observed in 20–50 cm soil layers. However a decrease in soil EC was recorded in the upper 0–10 cm soil layer.

Soil exchangeable sodium percentage (ESP)

Plants of *S. baryosma* were not able to reduce ESP and an increase in ESP was recorded in all depth levels. Maximum net increase in ESP was recorded in the upper 0–10 cm soil layer. In *S. nudiflora* planted plots, soil ESP decreased considerably in 0–40 cm depth soil layers. However plants failed to reduce soil ESP in 40–50 cm soil layer and increase in soil ESP was recorded for this depth level. In *H. recurvum* plots, a remarkable decrease in soil ESP was recorded in all depth levels.

Soil exchangeable sodium and calcium

An increase in soil exchangeable Na^+ was recorded in *S. baryosma* plots (Tab. 3). Maximum increase in soil exchangeable Na^+ was recorded in the upper 0–10 cm soil

layer. In *S. nudiflora* grown plots, the amount of exchangeable Na^+ was markedly reduced in the upper 0–10 cm soil layer. In other soil layers, i.e., 10–50 cm depth, an increase in the amount of exchangeable Na^+ was recorded. However, in 20–30 and 30–40 cm soil layers, only marginal increases in soil exchangeable Na^+ were recorded. In *H. recurvum* grown plots, the amount of exchangeable Na^+ was reduced considerably in all depth levels. Maximum reduction in exchangeable Na^+ was recorded in 20–30 cm soil layer. In control plots, an increase in exchangeable Na^+ was noticed in all depth levels. In *S. nudiflora* plots a significant increase in soil Ca^{2+} content was recorded at all depth levels. Soil Ca^{2+} content in *H. recurvum* plots increased significantly in the 0–30 cm soil layer. In the 40–50 cm depth level a marginal increase in soil exchangeable Ca^{2+} was also recorded. However in the 30–40 cm depth level a decrease in soil Ca^{2+} content was noticed. In *S. baryosma* grown plots, soil exchangeable Ca^{2+} content increased only in the 0–10 cm soil layers. However a significant decrease in soil exchangeable Ca^{2+} content was recorded in 10–50 cm soil layers.

Discussion

Our findings demonstrated that all three species of halophytes can be utilized as 'primer plants' and for phyto-remediation of secondary salinized agricultural fields. All three species had a clear modulatory effect on different soil physical and chemical properties.

Plants may influence the soil physical properties like pH and EC [14] and by that may counter the effect of salinity/alkalinity. *H. recurvum* and *S. nudiflora* were superior when compared to *S. baryosma* in reducing EC. The electric conductivity of the soil extracts of all the plots was far above the electric conductivity values of those found in Central Europe [15]. However, after plantation with halophytes these values can be reduced. Similar results for reduction in soil relative electric conductivity (REC) of saline-sodic soil (mainly above a 45 cm depth) by *Echinochloa stagninum* was reported by Helalia et al. [5]. Positive results for reduction of total soluble solids (TSS) by plantation of halophytic species *Juncus acutus* and *Juncus rigidus* were reported by Zahran et al. [7]. The improvement in soil permeability due to root action may also facilitate leaching, which in turn causes reduction in the EC of the upper soil layers. The soil pH is directly affected by the concerns of plant roots to H^+, OH^-, $HCO_3{}^-$, and organic anions reactions at soil exchangeable complex in response to disequilibria in cation and anion uptake effective under any particular soil system [16]. In addition to root respiration, root exudates, increased microbial activity and organic matter added by vegetation may alter the soil solution quality, which may influence the soil pH. Release of acidic root exudates decreases the soil pH [17]. Furthermore, $NH_4{}^+$ uptake by the plants may also reduce the soil pH significantly [18, 19]. Reduction in soil pH by growing *Leptochloa fusca* has been reported by Qadir et al. [6]. Extrusion of H^+ ion from roots, following ion transport mechanisms, is a general phenomenon in plant roots under a saline environment [20] and may contribute significantly to reduction in pH. In the present investigation, soil pH was considerably modulated by the planted halophytes. The effect of these plants on soil pH was variable at different depth levels. Although dependence of soil pH

upon different depth levels under different conditions like agriculture, forestry and natural conditions have been clearly established [16, 21], it may also be attributed to different root morphologies of the different plants investigated. The more accentuated influence of *H. recurvum* could be related to its deep reaching root system as compared to shallower root systems of *S. nudiflora* and *S. baryosma*. Chaudhary et al. [22] discussed the ability of *Suaeda fructicosa* to accumulate Na^+ and other ions. A single plant of *Suaeda fructicosa* may accumulate some 100 g of salt in its aerial tissue. In the present investigation, maximum amount of Na^+ was accumulated during a period of 3 months by *H. recurvum* followed by *S. nudiflora* and *S. baryosma*. Reduction in sodium content at the 20–30 cm depth level by plantation of *Suaeda salsa* plants has been reported by Zhao [10]. The root action of halophytes may mobilize the native lime of soil. Robbins [23] reported that CO_2 released during root respiration might be a major contributing factor for the reclamation of salt affected lands. Formation of $Ca(HCO_3)_2$ from $CaCO_3$ may also take place in the presence of H_2CO_3 resulting in increased solubility of $CaCO_3$ [24]. The Ca^{+2} thus released may replace the Na^+ from the exchange sites of the soil colloidal complex. The replaced Na^+ together with excess salts may be washed away from the root zone by rainwater or by any other source. In the present investigation *S. nudiflora* was the most efficient plant in increasing soil exchangeable calcium content. Further concept of biopores, i.e., pores left behind in the soil after death or decay of halophyte roots, may also be quite conceivable here [25]. Although such pores are in the macropore category ($> 100\mu$m diameter) and are created by thick roots, they are large enough to provide channels for optimum water and air conduction [25]. Consequently, roots of halophytes may alter several soil physical and chemical characteristics. As salt affected soils are generally degraded structurally and have a low permeability, growing halophytes may improve such concerns regarding soil–water and soil–air relations. All three halophyte species had a considerable impact on soil quality. The overall efficiency of these halophytes in reclamation was in the decreasing order of *H. recurvum* > *S. nudiflora*> *S. baryosma*. Plantation of these halophytes considerably altered the soil pH, EC, exchangeable Na^+, exchangeable Ca^{2+} and exchangeable sodium percentage (ESP). However, variability at different depth levels needs closer examination and continuous study. Investigations of soil structure and a closer characterization of differences in the root system of the three species should be further investigated. Halophytes may accelerate the reclamation process of salinization that otherwise would be unproductive or poor in yield. More than 1 year of intercropping will certainly be required to reclaim such soils and success surely will be influenced by the efficiency of rainfall. However, planting halophytes could replace fallowing for 1 or 2 years, as practiced presently, bringing more benefits for saline soils.

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Biosaline Agriculture and Salinity Tolerance in Plants
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Effects of irrigation water salinity on yield and evapotranspiration of drip irrigated cucumber in a semiarid environment

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Introduction

The major limiting factor on the expansion of irrigated agriculture throughout the world is the lack of water. Water demand is increasing due to fast population growth rates, improvement in living standards, improvement in industry and municipality, and global warming. Nowadays, there is an increasing tendency to use saline irrigation water in arid and semi-arid regions of the world because of rising water demands for irrigation. Slightly and moderately saline water can be used for irrigation successfully to grow salt tolerant and moderately salt-tolerant crops without adverse long-term effects on soil provided appropriate soil water management practices are followed [1].

Crop evapotranspiration (ET) under standard conditions applying different soil moisture regimes were studied but rarely under saline irrigation water. In general, salinity and drought affect the plant in a similar way. With increasing salinity or drought, soil water availability decreases. However, a question has arisen whether the yield-ET model developed under drought conditions can be valid under salinity conditions.

Stewart et al. [2] demonstrated that the relationship between yield and ET of maize was the same in cases of drought and salinity. Katerji et al. [8] checked this hypothesis for sunflower, potatoes and soybean in Italy using saline water. They reported that the hypothesis developed by Steward et al. [2] was valid for sunflower and, to a lesser degree for potatoes, but not for soybean. Shalhevet [3] reported that crop water production functions relating yield to evapotranspiration are not influenced by water salinity. Shalhevet and Hsiao [4] studied the effects of salinity and drought on cotton and pepper. They concluded that, at the same soil water potential, plants grown under saline conditions showed better growth than under drought. It seems that the yield response to ET due to salinity or drought is still a controversial subject.

The yield-ET relationship developed by Stewart et al. [2] was:

$$\left(1 - \frac{Y_a}{Y_m}\right) = k_y \left(1 - \frac{ET_a}{ET_m}\right) \tag{1}$$

Where: Y_a = actual harvested crop yield, Y_m = maximum harvested crop yield, k_y= yield response factor, ET_m = maximum ET, ET_a = actual ET.

Cucumber (*Cucumis sativus*) is considered to be a moderately salt-sensitive crop. Most of the studies in the literature have been conducted for determining the effect of saline water application on fruit quality and yield of field crops, vegetables and orchards rather than cucumber. On the other hand, the response of cucumber under saline irrigation water has not been adequately characterized. This research was conducted: 1) to check the hypothesis predicting yield from ET under saline conditions with models developed for drought conditions, 2) to quantify crop growth and yield of cucumber when grown under different irrigation water salinity levels, and 3) to determine threshold EC of irrigation water for drip irrigated cucumber.

Materials and methods

Plant culture, treatments and irrigation

The experiment was carried out on a clay loam soil, classified as Ikizce soil series (*Vertic Calciorthid aridisol*), from April–July 2001 in Sanliurfa, Turkey. The altitude, latitude and longitude of the experimental site are 465 m, 37°08'N and 38°46'E, respectively. The weather is hot and dry from May to September, when temperatures can reach up to 46°C. The 0–60 cm depth of soil profile had a dry bulk density of 1.32 g/cm^3, pH of 7.1, and EC_e of 1.0 dS/m. The field capacity (FC) was 32.50 %, and the permanent wilting point (PWP) 21.60 %, as determined gravimetrically. Irrigation water was of good quality with EC_i of 0.45 dS/m, containing (meq/L) 1.1 Ca^{2+}, 1.0 Mg^{2+}, 0.25 Na^+, 0.02 K^+, 0.75 $SO_4{}^{2-}$, 0.90 $HCO_3{}^-$, 0.60 Cl^- and a pH of 7.0.

A hybrid cultivar 'Beith Alpha F1' of cucumber widely cultivated in southeast Turkey was selected. Seeds were germinated in fine sand during the second week of March and at the second true leaf stage (20 days) similar sized seedlings were transplanted into plastic tubs containing previously washed sand. Similar sized seedlings were again selected at the fourth true leaf stage (12 days) and transplanted to the field in the second week of April. The plants were drip irrigated according to their scheduled program after transplanting.

33 plants per replicate were planted in rows with an inter-plant spacing of 0.5 m and an inter-row spacing of 1.0 m. A single drip irrigation tube (Goktepe Co., Izmir, Turkey), with 4.0 L/h and 0.5 m emitter spacing, was placed for each row. Each experimental plot was composed of three 5 m long rows. Yield was measured only for plants growing in the central row, the outer rows serving as borders. The operating pressure of the drip irrigation system was constant during the experiment as 100 kPa. Each plot had a separate flow meter (Teksan Co., Turkey) to monitor water input.

All treatments received the same amounts of total N (12 kg/da), P_2O_5 (15 kg/da)and K_2O (24 kg/da) fertilizers. Based on soil test results, all of the P as a diammonium phosphate (18-46-0) was applied prior to planting and mixed into the soil. The N as ammonium nitrate (33 % N) and K as potassium nitrate (13-0-46) were added equally at weekly intervals through the drip irrigation system, starting

after transplanting until the second harvest. Hand weeding was carried out 3 times during the growing season.

Treatments were: 1) fully irrigated with good quality water of 0.45 dS m^{-1} EC_i (C), 2) fully irrigated with saline water of 3 dS m^{-1} EC_i (SW_1), 3) fully irrigated with saline water of 6 dS m^{-1} EC_i (SW_2), and 4) fully irrigated with saline water of 9 dS m^{-1} EC_i (SW_3). All treatments were drip-irrigated every 3 days. Saline solutions were obtained by adding NaCI to irrigation water to obtain the EC_i levels of 3, 6, 9 dS m^{-1}.

Evapotranspiration (ET) for each plot was calculated according to the water balance approach. In order to determine the actual ET, soil moisture content between 0 and 90 cm was measured gravimetrically prior to irrigation.

Plant growth

Plant growth was monitored using leaf area measurements. Randomly chosen three whole plants from each plot were sampled at the end of the first harvest. Total leaf area was determined with a portable leaf area meter (LI-3100, LI-COR, Lincoln, NE). The harvesting was initiated in the first week of July. Fruits were harvested every 2–3 days depending on fruit size until the end of July. Leaf relative water content (LRWC) was calculated based on the methods from Yamasaki and Dillenburg [5].

Crop salt tolerance and statistical analysis

The yield response to salinity was evaluated by a linear regression model (threshold-slope model) proposed by Maas and Hoffman [6]:

$$Y_r = 100 - s(EC_i - EC_t) \tag{2}$$

Where Y_r is the percentage of the yield of the crop grown under saline conditions relative to that obtained under non-saline (control) conditions, the EC_t is the threshold salinity value tolerated by the crop without yield loss, the s is the yield loss per unit increase in salinity (or shortly, slope of the regression between relative yield and EC_e).

Mass and Hoffman [6] summarized the relationship between salinity and yield as:

$$Y_r = \left\{ \begin{array}{cc} 100 & 0 \leq ECe \leq EC_t \\ 100 - s\,(EC_e - EC_t) & EC_t \leq ECe \leq EC_0 \\ 0 & ECe \geq EC_0 \end{array} \right\} \tag{3}$$

Where EC_0 is the level of salinity at which the yield is zero.

The layout of the experiment was in a randomized complete-block design with three replications. The data were subjected to ANAVO and Duncan's least significant difference (LSD) test to check the significance.

Results and discussion

Evapotranspiration and yield

The yield and ET values of each treatment are presented in Table 1. Figure 1 shows the measured and estimated yield of cucumber. The yield estimation of cucumber is very good since the slope and the intercept of the regression line is not significantly different from 1 and 0, respectively. Equation 1 tends to overestimate yield for SW_1 and SW_2, but underestimated yield for SW_3. However, both overestimation and underestimation never exceeds 10 % within the range of measured yields. The linear regression analysis between measured and estimated yields was Y(estimated) = 1.07 * Y (actual) – 0.086 with an R^2 of 0.98. The results of this study confirm those of Stewart et al. [2] on maize, Katerji et al. [1] on sunflower and potatoes and support their conclusion concerning a similar relationship between yield and ET for both drought and salinity.

Table 1. Effects of irrigation water salinity on fruit yield, ET and plant growth of drip irrigated cucumber grown in semiarid conditions

Tr.	App. water (mm)	ET (mm)	Total yield (kg da^{-1})	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Fruit no per plant	Leaf area (cm^2)	LRWC (%)
C	825	841	3187 a*	17.5 a	4.5 a	235 a	15.2 a	1875 a*	91.1 a
SW_1	796	795	3067 a	16.2 a	4.4 a	222 a	14.8 a	1827 a	89.2 a
SW_2	714	680	2311 b	9.4 b	2.9 b	166 b	6.1 b	1101 b	70.2 b
SW_3	402	338	915 c	5.8 c	2.8 b	76 c	1.6 c	412 c	52.5 c

*Within each column, means followed by the same letter indicates no significant difference between treatments by Duncan's multiple range test at $P \leq 0.01$

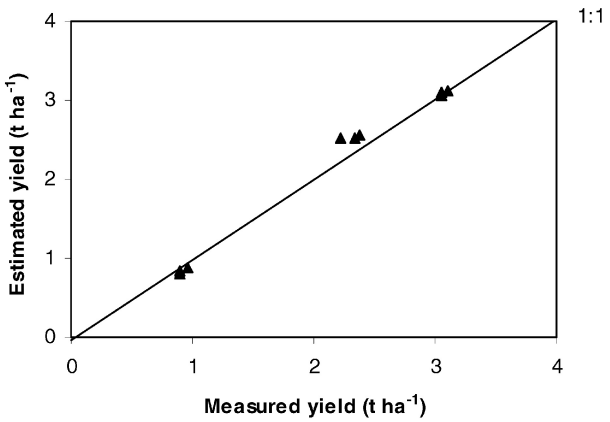

Figure 1. Measured yield of cucumber *versus* yield estimated with Equation (1)

Saline irrigation caused an increase of the soil water content due to increased osmotic potential of soil nutrient solution. While the ET of the control treatment was 841 mm, saline treatments SW_1, SW_2 and SW_3 had a 795, 680 and 338 mm of ET, respectively. This is in agreement with the findings of Sonneveld and Voogt [7], who reported that increasing irrigation water salinity reduces transpiration and increases drainage for a given irrigation volume.

Applied water for each treatment was different since irrigation was scheduled to increase soil moisture to field capacity. The applied water and ET for C treatment was 825 and 841 mm, respectively. As the EC of irrigation water increased, the applied irrigation water decreased due to reduced ET. Reduction in ET in the presence of salinity is often partially caused by reduced plant size and fraction of ground cover. The difference observed in ET is a reflection of differences in transpiration. The saline conditions in the root zone hinders water uptake because of reduced osmotic potentials caused by increased salinity, which ultimately decreases transpiration of the crop. The changes among EC of irrigation water, yield and ET are shown in Figure 2. Figure 2 shows that there was a similar trend of reduction for both ET and yield with increasing irrigation water salinity.

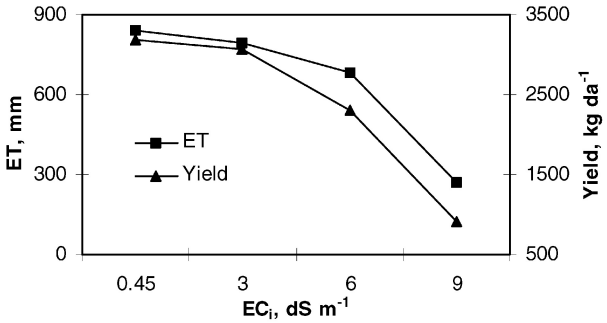

Figure 2. Relationship between EC_i, yield and ET

Drip irrigation systems helped to maintain higher soil moisture, resulting in higher transpiration. High water content may alleviate inhibition in water uptake caused by salinity. However, this situation is valid to a certain point. Under severe EC_i conditions, crops cannot make necessary internal adjustment of osmotic potential, so ET and plant growth decreased sharply.

The average fruit yield was greater at the control and 3 dS m^{-1} EC_i. At 6 and 9 dS m^{-1} EC_i, yield was reduced by 27.5 % and 71 %, respectively. Both fruit weight and fruit number were reduced significantly with increasing salinity. An average of 60 % and 89 % fewer fruits were harvested at 6 and 9 dS m^{-1} EC_i, respectively, compared to the control treatment. The individual fruit weight at the same irrigation water salinity levels was reduced 29 % and 68 %, respectively, compared to the control treatment.

Plant growth

The decline in cucumber vegetative growth at high salinities was expressed as reduced total leaf area. It was found that the maximum leaf area resulted from the lowest water salinity. As the irrigation salinity levels increased, plant growth (leaf area) decreased linearly (Tab. 1). These observations are similar to the results for cotton presented by Vulkan-Levy et al. [8]. Total leaf area reduced an average reduction of 3 %, 41 %, and 78 % with salinity levels of 3, 6 and 9 dS m^{-1}, respectively.

The LRWC decreased with increasing salinity of the irrigation water (Tab. 1). The LRWC at waters of 6 and 9 dS m^{-1} EC_i was decreased 23 % and 42 %, respectively, compared to C. This result is in agreement with the findings of Katerji et al. [9] and Maggion et al. [10].

Crop salt tolerance

The threshold irrigation salinity value $EC_{i(t)}$ for drip irrigated cucumber was found at 3.4 dS m^{-1} and the $EC_{i(0)}$, which the yield is zero, was 12.3 dS m^{-1}. The slope (s) was 11.17 % per dS m^{-1} (Fig. 3). The yield response to irrigation salinity was calculated as Yr = 100 – 11.17 (EC_i- 3.4).

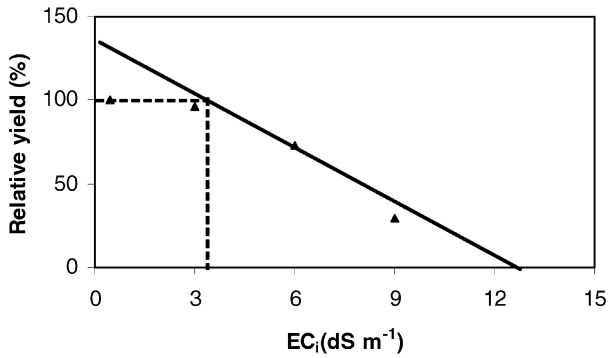

Figure 3. Relative yield response of drip irrigated cucumber to increasing salinity of irrigation water

According to Ayers and Westcot [11, $EC_{i(t)}$, $EC_{i(0)}$, and s values for cucumber were 1.7, 6.8 dS m^{-1} and 19.5 % per dS m^{-1}, respectively. We calculated that threshold and zero yield values of cucumber were bigger than that of Ayers and Wescot [11], whereas the slope value was lower. These differences could be due to drip irrigation systems which absolutely have some advantages in the use of low quality water. Secondly, we conducted the experiment over only 1 year. The negative effect of the saline irrigation water would occur potentially in the next year. Chartzoulakis [12] found that the threshold irrigation salinity for greenhouse grown cucumber was 1.3 dS m^{-1}. The researcher also noted that each unit of EC greater than the threshold decreased yield by 15.9 %.

High temperature and low relative humidity are two main characteristics of arid climate and actually these two parameters increased the ET and likely intensed the negative effect of salinity on crop growth. The arid and semi-arid conditions tend to lower the crop's threshold for salinity stress either because of high transpiration or changes in leaf biochemistry [13]. However, our results showed the opposite of this idea. The likely reason of it could be the irrigation method chosen.

The threshold soil salinity value $EC_{e(t)}$ for drip irrigated cucumber was found to be 1.5 dS m^{-1} and the $EC_{e(0)}$, which the yield is zero, was 8.85 dS m^{-1}. The slope (s) was 13.6 % per dS m^{-1} (Fig. 4). The yield response to soil salinity was calculated as $Yr = 100 - 13.6\ (EC_e - 1.5)$. Allen et al. [14] reported $EC_{e(t)}$ between 1.1–2.5 dS m^{-1} and slope between 7–13 % per dS m^{-1} based on climate, soil conditions and cultural practice. Although our EC_e threshold value was between reported values of Allen et al. [14] , the slope value was a little bit higher than that of Allen et al. [14].

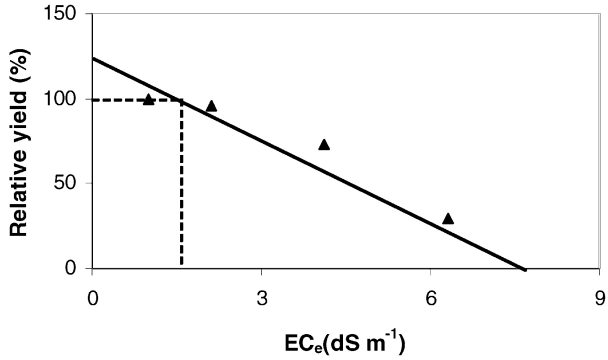

Figure 4. Relative yield response of drip irrigated cucumber to soil salinity

The experimental results showed that the EC_i threshold value was bigger than the EC_e threshold. This result is in agreement with the findings of Bahceci [13]. In similar soil and environmental conditions in Konya-Turkey, Bahceci [13] reported that EC_i(4 dS m^{-1}) threshold value of sprinkler irrigated bean was higher than that of EC_e(0.81 dS m^{-1}). The researcher explained this situation with low initial soil salinity. In our experiment, it was most probably caused by both low initial soil salinity and the chosen irrigation method itself.

Conclusions

The use of drip irrigation systems in semi-arid regions shows a great potential to use the low quality irrigation water in irrigation with necessary precautions. Although the experiment period was only 1 year, the results obtained provided useful insights into the effects of salinity on yield, growth and ET. If the soil is not saline, irrigation water salinity up to 3 dS m^{-1} would not significantly affect the yield of drip irrigated cucumber. However, irrigations with low quality water above 3 dS m^{-1} reduced the

yield significantly. In particular, the negative effect of the SW_3 treatment was much more severe compared to other saline treatments.

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Biosaline Agriculture and Salinity Tolerance in Plants
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Potential utilisation of halophytes for the rehabilitation and valorisation of salt-affected areas in Tunisia

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Introduction

In arid and semi-arid regions, irrigation water contributes to salinisation of the upper layer of the soil, where most root activity takes place. Along the path of plant domestication, many crop species have lost resistance mechanisms to various stress conditions [1], including salt stress [2]. Thus, most crop plants do not fully express their original genetic potential for growth, development and yield under salt stress, and their economic value declines as salinity levels increase [3, 4]. Improving salt resistance of crop plants is, therefore, of major concern in agricultural research. A potential genetic resource for the improvement of salt resistance in crop plants resides among wild populations of halophytes [5, 6]. These can be either domesticated into new, salt-resistant crops, or used as a source of genes to be introduced into crop species by classical breeding or molecular methods.

Given the progressive scarcity of freshwater resources and soil salinisation, a major aim of investigations is to evaluate the potential of halophytic species to be widely and economically used in arid and semi-arid regions. It would encourage the sustainable use of halophytes for the creation of productive ecosystems and re-greening degraded areas, by building up a collection of halophytes with a high tolerance to salt stress and characteristics potentially exploitable from an economic point of view. Among the known 2,600 halophytic species, some present economic (human feeding, fodder, materials of high economic values) or ecological interests (soil desalinisation, dune fixation, phytoremediation, landscaping and ornamentation).

Within the framework of this approach, the Laboratory of Plant Adaptation to the Abiotic Stresses, in the National Institute of Scientific and Technical Research (INRST) of Tunisia has initiated an exploration and a physiological and biochemical characterisation of some halophyte species in order to identify the most promising

ones. There are two major topics: the implication of halophyte species in the improvement of soil characteristics (desalinisation and fertilisation, heavy metal extraction), and their economical interests as oleaginous and fodder crops, for instance.

Results

Ecological interests of halophytes

Improvement of soil characteristics

Vegetation in saline habitat such as sebkha is heterogeneous. Numerous perennial tufts of strict halophytes are associated with annual species sensitive to salt and mineral deficiency stresses. *Medicago*, characterised by a high fodder value, largely contributes to the ecosystem primary production in the absence of water constraints. These annuals mainly develop within or very close to halophyte tufts. Parallel field and laboratory studies have shown that *Medicago* is sensitive to salinity [7, 8], as well as to nitrogen and phosphorus deficiencies [9]. Furthermore, the shoots of the annuals growing in association with halophyte species contains relatively low Na^+

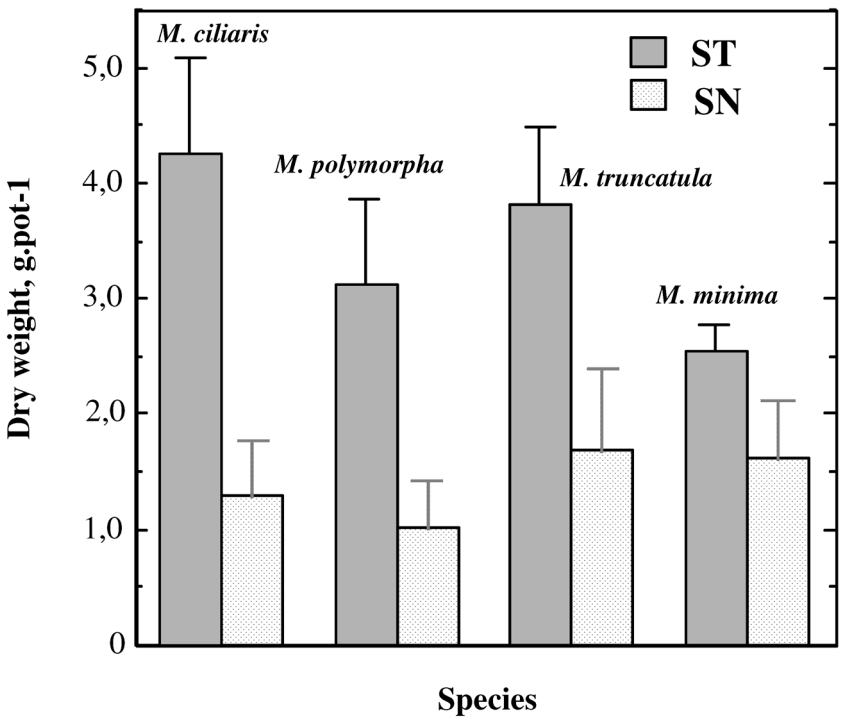

Figure 1. Changes in biomass production of *Medicago ciliaris*, *M. polymopha*, *M. truncatula* and *M. minima* ($g.pot^{-1}$) with soil origin. ST: culture carried out on soil sampled under halophytes tufts, SN: cultures carried out on soil sampled in outside of halophytes tufts. Means of 20 repetitions and confidence intervals at 95 %

concentrations. These data suggest that the upper horizon of spots near the halophyte tufts (where sensitive annuals grew), is fertile and contains low salt levels. Indeed, this was confirmed by the study of soil samples taken from the upper horizon in the tuft centres. Desalinisation of the upper horizon by the superficial roots of halophytes could be responsible for this microgradient of salinity. Moreover, the litter formed by halophyte fallen organs and by organic debris accumulated by the wind at the vicinity of halophyte tufts, could contribute to localised soil enrichment in N and P. This was confirmed using a biological test of soil fertility. Some tufts of *Salicornia arabica* were removed for sampling soil in the upper horizon (0–20 cm), where roots of annual plants developed. Other samples were taken between halophytes tufts, in zones devoid of vegetation or weakly populated. Four annual *Medicago* species (*M. ciliaris*, *M. polymorpha*, *M. truncatula* and *M. minima)* were grown on these soil samples, without mineral fertilisation, in a greenhouse under controlled conditions. The plants were harvested at the flowering stage. In the four species, total biomass production (dry matter per pot) was higher on soils sampled under the halophyte tufts than on soils from nude zones (Fig. 1). These studies show that perennial halophytes improve soil characteristics by lowering its salt content and by increasing nitrogen and phosphorus concentrations.

The capacity of desalination of saline soil by halophytes was also evaluated in strictly controlled conditions, using *Sesuvium portulacastrum*, an Aizoaceae. After clonal multiplication, the plants were cultivated for 2 months on saline soil, originating from the edge of a sebkha. They were irrigated with a nutrient solution deprived of $Na^+$and Cl^-, without losses by drainage. Salt export by plants was evaluated by the difference between the quantities of Na^+ and Cl^-, initially measured in the culture

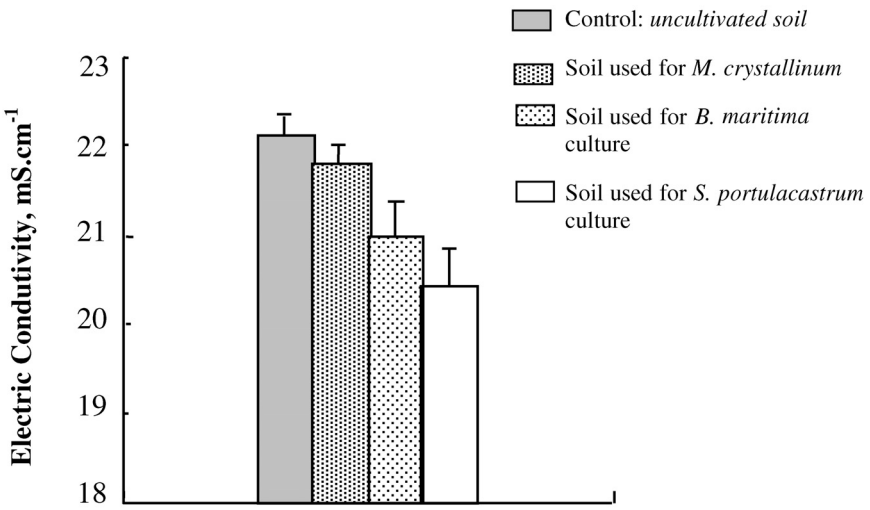

Figure 2. Electric conductivity ($mS.cm^{-1}$) of the aqueous extract (1/10) of soil used during two months for halophytes culture. Means of 12 replicates. Bars indicate ± standard errors ($p = 0.05$)

substrate, and those found at the end of the experiment in the soil. *S. portulacastrum* produced more biomass than other species used in the same experiment (*Batis maritima* and *Mesembryanthemum crystallinum*), and accumulated larger amounts of Na^+ and Cl^-(about 6.5 mmol.g^{-1} DW, amounting to 30–40 % of the biomass). At the end of the cultures, the soil used for the culture of *S. portulacastrum* showed a significant (10%) decrease of its salinity estimated by electric conductivity (Fig. 2). This study demonstrates that the associated characteristics of *S. portulacastrum*, namely high growth rate and high capacity for salt accumulation, permit soil desalination, even in short-term cultures. Thus, this species would be interesting for the rehabilitation of the saline lands.

Heavy metal extraction

In Tunisia, saline depressions, colonised by halophyte species, often constitute sites of accumulation of industrial effluents contaminated by heavy metals. Indeed, preliminary studies achieved in various regions of Tunisia showed that these zones are contaminated by cadmium, nickel and lead. We studied the response to Cd of two halophyte species, *S. portulacastrum* and *M. crystallinum*. In the absence of Cd, the biomass of *M. crystallinum* plants was much larger than that of *S. portulacastrum*. However, Cd severely inhibited *M. crystallinum* growth, even at the lowest concentration (50 μM), but did not significantly modify that of *S. portulacastrum.* In the shoots, the Cd concentrations in *S. portulacastrum* shoots was half (100–350 ppm)

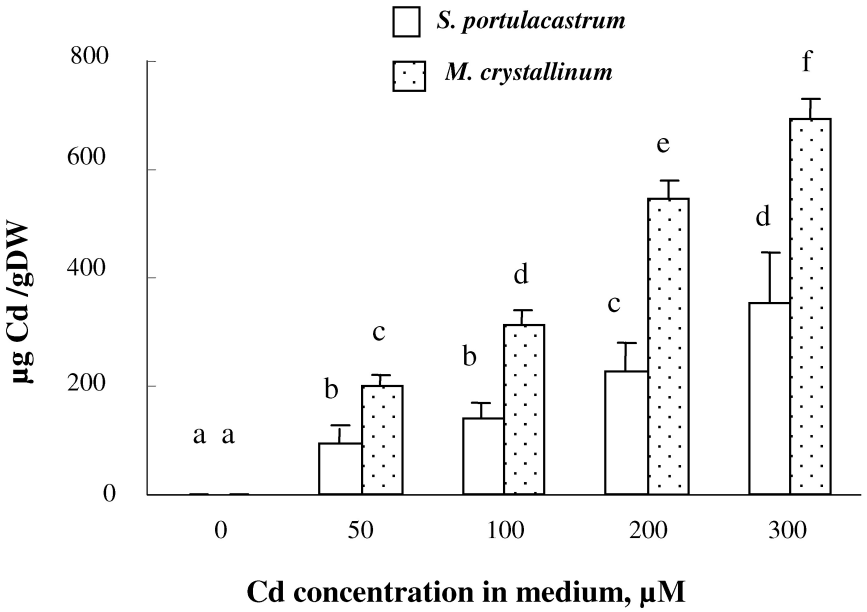

Figure 3. Changes in Cd concentration (μg.g^{-1} DW) in shoots of *S. portulacastrum* and *M. crystallinum* treated by various Cd concentrations. Means of 8 replicates. Bars marked with same letter are not significantly different at p = 0.05

that in *M. crystallinum* shoots (200–700 ppm) (Fig. 3). According to these data, both species would be classified among Cd hyper accumulator plants and would be of this fact interesting for the phytoremediation. The analysis of the relationship between growth and mineral status in the two halophytes suggested that the Cd-induced decrease of growth resulted not from direct effect of accumulated Cd, but rather from restriction of K^+, Ca^{2+}, and Fe^{2+} uptake. This hypothesis was studied using a split-root system: after a pretreatment phase, seedlings were divided into three lots. Half of the roots of the first lot were immersed in Cd free medium, while the other half were immersed in the same medium supplemented with 100 μM Cd (B/Cd). For the two other lots, the two halves of the root system were immersed either in free Cd medium (B/B) or in medium supplied with 100 μM Cd. In comparison with Cd/Cd plants, the split root Cd/B plants displayed improved growth. This effect was associated to an increase in nutrient uptake. Furthermore, the Cd/B plants accumulated Cd at a level similar to that of Cd/Cd plants. In summary, our results indicate that the Cd-induced decline growth resulted rather from an indirect Cd effect (inhibition of nutrient uptake) than from a direct Cd effect (excess of Cd accumulation) and suggest the possibility to increase the capacity of the two halophyte species to extract Cd while improving nutrient availability in the medium.

Economical interests of halophytes

Halophytes with fodder potential

In Tunisia, fodder crops occupy currently only 7 % of the cultivated zones. In addition, the surface of the uncultivated saline area is four times more important than that of the pastures. The identification of fodder halophytes would make it possible to exploit new zones of production and to reduce our deficit in fodder. Within the framework of this approach, several potentially suitable species were identified. The conditions of their multiplication and culture were established as well as their salt tolerance limits and their nutritional requirements.

Suaeda fruticosa, an indigenous Chenopodiaceae in Tunisia, is quite frequent in semi-arid, arid and desertic bioclimatic stages and well appreciated by livestock [10]. *Spartina alterniflora*, a Poaceae, is dominant in saline marsh and coastal regions in the east of USA. For *S. fruticosa*, maximal dry matter production occurred at NaCl concentrations comprised between 100–300 mM, with a 85-fold increase in dry weight following a 45 day treatment. *S. alterniflora* expressed maximal growth when irrigated with nutrient solution or containing 0–100 mM NaCl, its initial dry weight being six-fold increased after 100 days of treatment. *S. fruticosa* was more salt tolerant than S. *alterniflora* under moderate NaCl concentrations (300 mM NaCl), but more sensitive at the highest NaCl concentration (800 mM). Considering the decreasing availability of freshwater in arid regions, the utilisation of non-conventional water resources (brackish water, waste water, and seawater) constitutes a promising approach, especially as these halophytes require salt to express their maximal growth potentialities. However, the growth of both species was limited when they were irrigated with seawater, owing to the low availability of some nutrients. Indeed, they displayed higher growth rates when nitrogen and, to a lesser degree, phosphorus were added to the seawater [11].

The capacity of biomass production is an important characteristic which must be considered in the evaluation of the fodder halophytes. Using cultures carried out in pots of 0.85 m^3, we demonstrated that *S. alterniflora* could produce 7,500 kg DW per hectare in one cut. As the season of growth of this species extends from March–October, it is possible to carry out at least two cuts, which will ensure a primary production of 15 tons DW per hectare and per year. Similar estimation showed that *S. fruticosa* could produce at least 4,500 kg DW per hectare. These yields are comparable to those of the conventional fodder crops irrigated with freshwater, like alfalfa, 10 tons.ha^{-1} per year [12], and clover, 8 tons.ha^{-1} per year [13]. Analysis of published data indicates that the yield of the most productive halophytes varies from 8–17 tons DW.ha^{-1} per year. The comparison of the mineral composition of the shoots of both halophytes with that of the fodder required by livestock showed that excepting Ca^{2+} content in *S. alterniflora*, K^+, Mg^{2+} and P concentrations in tissues exceed the nutritional requirements of the cattle. The content of total nitrogen is higher in *S. fruticosa* than in *S. alterniflora*, but the digestible nitrogen fraction is similar in both halophytes, and meets perfectly the nutritional requirements of the sheep. According to Glenn and O'Leary [14] and Bayoumi et al. [15], proteins represent 15 % of DW in several halophytes. According to these data, *S. fruticosa* and *S. alterniflora* can annually produce 1.5 tons of proteins for an average biomass of 10 tons.ha^{-1}. The quantitative and qualitative yield of the two halophytes is appreciably similar to that of alfalfa irrigated with freshwater [16].

Two other halophytes from Tunisia, with fodder potential, were characterised: *Aeluropus littoralis* and *Catapodium rigidum*, respectively perennial and annual Poaceae. In the absence of salt, *Catapodium* displayed a relative growth rate (RGR) slightly higher than that of *Aeluropus*. Salt decreased this parameter in both species. On RGR basis (expressed as % of control without salt) *A. littoralis* was more tolerant than *C. rigidum*, this behaviour being accentuated with increasing salinity. In salinity range not exceeding 400 mM, RGR remained between 0.04–0.06 day^{-1}, values characteristic of spontaneous or cultivated *Medicago* species not subjected to salt, 0.08–0.09 [7, 9] or others fodder halophytes *Suaeda fruticosa*, 0.07–0.09 [11], *S. alterniflora*, 0.03 [11], *Spartina anglica*, *Puccinellia maritima*, 0.02–0.05 day^{-1} [17].

Both halophytes accumulated sodium mainly in their shoots. However, Na^+ concentrations were lower in *A. littoralis* than in *C. rigidum* (maximum values around 5 mmol. g^{-1} DW and 10 mmol. g^{-1} DW, respectively). In addition to its capacity to control Na^+ transport towards shoots, *A. littoralis* secreted more than 50% of leaf Na^+ by salt glands. Indeed, Na^+ accumulated inside tissues did not exceed 2 mmol.g^{-1} DW under the most severe salinity. This secretion seems to be selective for Na^+ and Cl^-, since K^+ was completely absent among the elements secreted on the surface of the leaves.

Oleaginous halophytes

Some Tunisian salty areas have been explored for plants which could be considered as oilseed species. Three potentially interesting species have been identified as oil producers, *Zygophyllum album* (Zygophyllaceae), *Cakile maritima* (Brassicaceae),

and *Crithmum maritimum* (Apiaceae). Oil extraction was carried out on ripe seeds collected in their natural biotope. Some physiological and biochemical aspects were studied, such as individual mass of seeds, oil content, and lipid and fatty acid composition. The seeds of investigated species have a suitable size for harvesting. The dry weight of 100 seeds ranged from 133 mg in *Zygophyllum album* to 774 mg in *Cakile maritima*. The value for the latter species is nearly three times higher that for rape (*Brassica napus*) seeds, a conventional oleaginous plant. The seeds of the two other halophytes are smaller than *Cakile maritima* seeds, with mass approximately half of that of rape ones.

The seeds of *Cakile maritima* and *Crithmum maritimum* present high levels of oil, reaching respectively 42 % and 30 % of the seed DW. Oil content in *Zygophyllum album* seeds is very low (6 %). As for olive oil, fatty acid composition of *Crithmum maritimum* seeds is characterised by a high level of oleic acid (81 %), whereas that of *Zygophyllum album*, characterised by a high percentage of linoleic acid (64 %), is similar to sunflower oil composition. Therefore, these two species contain oils of good quality which can be used without any further modification. Oil of *Cakile maritima*, rich in erucic acid (25 %) may be used for industrial applications [18].

In the laboratory, growth of *Cakile maritima* was stimulated under moderate (50–100 mM) concentrations of NaCl. The response of the whole plant was essentially due to the salt effects on shoots, their growth being significantly augmented at 100 mM NaCl. In the 200–300 mM range, the whole plant biomass production was maintained at approximately 90 % of the control. At higher NaCl concentrations

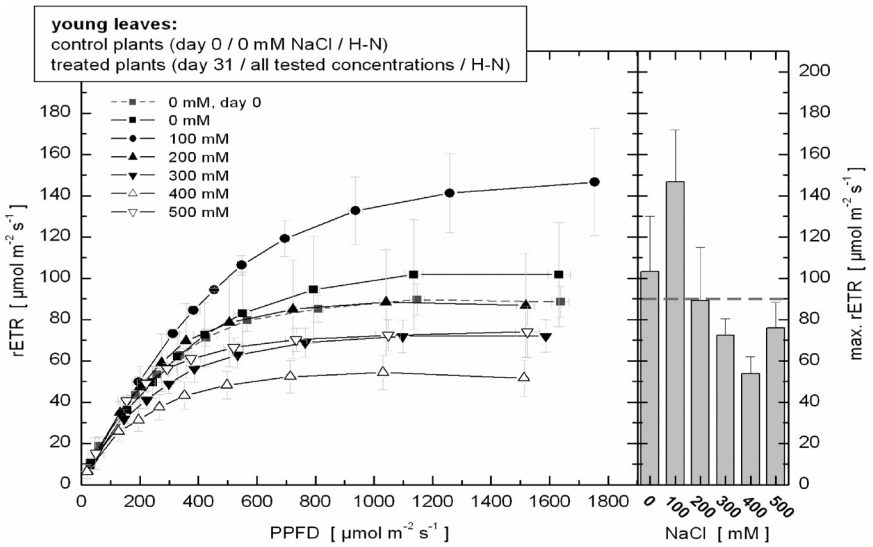

Figure 4. NaCl effect on photosynthesis. Changes in the electron transport rate (ETR) in the leaves of *Cakile maritima* subjected to increasing NaCl concentrations for 35 days. Means of 10 replicates ± standard errors ($p = 0.05$)

(500 mM), an important and significant reduction of growth was observed, but plants survived [19]. Concerning photosynthesis, various parameters were measured: gas exchange, net assimilation of CO_2, and electron transport rate (Fig. 4). As growth rate, these parameters indicated that the optimal physiological functioning was obtained with 50–100 mM NaCl. In these optimal conditions, the number of seeds per plant was significantly augmented by salt. At higher concentrations, a significant reduction of seed production was observed. All salt treatments resulted in seeds significantly smaller than in control. Culture of *Cakile maritima* in the presence of salt modified the biochemical composition of seeds: the oil content and the rate of erucic acid, largely used in industry, were augmented.

Conclusions

These studies were aimed at identifying among the halophytes promising species to ensure a plant productivity of economic and/or ecological interest, in the marginal zones and under conditions of irrigation with non-conventional water resources (brackish water, waste water and even seawater, more or less diluted). We identified several halophytes interesting for livestock nutrition. Monocotyledonous with a salinity avoidance strategy, enabling them to produce a biomass containing relatively little salt (*Spartina alterniflora*, *Aeluropus littoralis*, *Catapodium rigidum)* are particularly interesting. Culture conditions of these plants, their limits of salt tolerance and their nutritional requirements, are known. The data obtained so far are promising. Work is in progress to characterise their response to other constraints which could limit their yield in saline lands and particularly the low nitrogen availability [20]. Indeed, nitrogen is the most limiting nutrient for plant growth in saline ecosystems. Since a greater availability of NH_4^+ than NO_3^- was often observed, the research of the halophytes able to use ammoniacal nitrogen would be an effective and less expensive substitute than the use of nitrogen mineral fertilisers.

Two promising oleaginous halophytes were identified: *Cakile maritima* and *Crithmum maritimum*. An important intraspecific variability was observed within these two species at the levels of i) seed biochemical characteristics (oil content, fatty acid composition), and ii) the physiological response to salt. Studies carried out on these plants from germination to the seed maturation showed that *Cakile maritima*, producing industrial oil, is relatively more tolerant to salt than *Crithmum maritimum*, producing edible oil [21]. However, recent data showed that *Crithmum maritimum*, rich in antioxidant molecules, is also interesting for medicinal purposes.

At the ecological level, particular interest was paid to the halophytes able to fix and desalinate soil. *Sesuvium portulacastrum* with its growth stimulated by high (800 mM salt) concentration under adequate mineral nutrition is a promising candidate. The analysis of its responses to heavy metals also suggests that this species is interesting for phytoremediation.

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Biosaline Agriculture and Salinity Tolerance in Plants
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Interactive effect of potash and organic manures on growth and nutrient uptake of sugarcane grown under saline conditions

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Salinity and crop production

Soil salinity poses severe problems to crop production in many countries and this problem is very severe in arid and semi-arid regions of the world which occupy one third of the area of the earth (4.3 billion ha). Salinity is among the serious problems of irrigated agriculture of Pakistan. Millions of hectares of potentially productive land is uncultivatable due to excess of salt. The population of Pakistan is increasing at 2.61 % and the gap between the supply and demand of agricultural products is widening day by day. In order to meet the future demand of food, fiber, fuel and industrial raw material, the extension of agriculture would require the use of marginal lands [1]. Most of Pakistan is arid to semi-arid and has a low annual precipitation. Of the 20.36 mha of the total cultivated land, 6.3 mha are salt-affected [1]. A major part of the salt affected soils (about 3.5 mha) are presently cultivated to rice, wheat, cotton and sugarcane, but the output is very low. According to an estimate, the reductions in the yield of rice, wheat, cotton, sugarcane cultivated on such moderately salt-affected soils are, 64, 62, 59 and 68 %, respectively [2].

Role of potassium in plant growth and metabolism

Potassium is an essential nutrient element for all plants and in most terrestrial plants K^+ is the major cationic inorganic nutrient element. Potassium acts to balance the charge in the cytoplasm of plant cells, where K^+ is the dominant counter ion for the large excess of negative charge on proteins and nucleic acids [3]. It activates the crucial enzymatic reactions such as those occurring in the formation of pyruvate and is also a substantial contributor to the osmotic pressure of the vacuole and hence to cell turgor which endows non-lignified plant cells with structural rigidity. In contrast Na^+ is only required for halophytes (for translocation of pyruvate across the chloroplast

envelope) where it acts as a micronutrient [4]. In most other species Na^+ does not act as a nutrient in the sense that it is strictly required for growth but its addition to the growth medium may promote growth of many plants when the K^+ supply is limited and particularly the growth of salt tolerant plants, by contributing to turgor formation [5].

Soil productivity and organic/inorganic amendments

A major agricultural research priority is to sustain soil productivity and to develop better methods to monitor changes in soil physical, chemical and biological properties as affected by soil management. It is well established that organic addition of amendments to soil can positively affect soil as well as crop productivity. An organic manure amendment to soil increases soil fertility and porosity, microbial biomass and microbial activity [6]. There are many reports, which indicate that application of organic matter increases soil urease and phosphatase activities and add N and organic matter to soil. Microbial degradation and mineralization of organic matter provides nutrients to become available to the growing crop [7]. The amendment of organic matter also increases the moisture holding capacity and the Ca^{2+} exchange capacity and decreases soil pH as a result of which micronutrients become available to plants [6].

Heavy yield losses were observed in different crops due to salinity. According to an estimate, the yield losses in sugarcane on moderately salt-affected areas were up to 62 % [8]. Different approaches are being used to utilize or to reclaim the salt-affected soils. Presently, we have planned to utilize the moderately salt-affected soils for sugarcane production by the management of Na/K ratio. Potassium is an essential major element involved in maintaining the water status of plant and turgor pressure of its cells and opening and closing of its stomata. Potassium is required in the accumulation and translocation of newly formed carbohydrates.

Keeping in mind the importance of organic matter and potassium for soil and plant productivity, experiments on four sites (one normal and three salt affected) of Punjab, Pakistan, are being conducted to investigate the relative response of sugarcane crop to SOP (K_2SO_4) versus MOP (KCl) with amendment of two types of organic manures in salt-affected soils.

Field studies and soil/plant analysis

The interactive effect of Potash, i.e., SOP or MOP, and two organic fertilizers, i.e., farmyard manure and sugarcane press mud, on the yield and quality of two sugarcane varieties, SPSG-26 (salt tolerant) and CP-77-400 (salt sensitive), was studied at three selected sites in Pakistan which are normal, saline and saline-sodic. The soil and water samples of three selected sites, i.e., NIAB Faisalabad, Jhang, and Samundri, have been collected and analyzed for various physicochemical properties, i.e., soil texture was determined by hydrometer method [9], water holding capacity estimated by Hillguard method, bulk density by core sampler technique (100 cm^3), permanent wilting

Table 1. Soil characteristics of four selected sites to study the effect of SOP and MOP on the yield and quality of sugarcane

Soil characteristics	**Selected sites**			
	NIAB	**JHANG**	**PINDI BHATTIAN**	**SAMUNDRI**
Soil Texture	Clay-loam	Sandy-clay-loam	Clay	Clay-loam
Ece (dS m^{-1})	1.03–1.732	10.09–19.99	4.7–10.8	5.26–16.75
PH	7.4–7.9	8.35–8.46	8.41–9.29	8.81–9.15
SAR	0.45–0.62	27.37–99.2	41–78	29–86
Na^+ (meq L^{-1})	0.9–1.2	75–130	80–150	60–110
Ca+Mg (meq L^{-1})	7.5–10.0	15.0–20.0	7.5–12.5	8.5–18.5
K^+ (meq L^{-1})	1–1.25	1.2–1.5	1–1.25	1–1.56
$CO_3{}^{2-}$ (meq L^{-1})	–	–	–	–
$HCO_3{}^-$ (meq L^{-1})	–	25–45	25–75	28–60

Table 2. Tubewell waters used for irrigation at four selected sites to study interactive effects of SOP, MOP and organic fertilizers (farmyard manure and sugarcane press mud) on the yield and quality of sugarcane

Tubewell water characteristics	**Selected sites**			
	NIAB	**JHANG**	**PINDI BHATTIAN**	**SAMUNDRI**
EC (dS m^{-1})	0.77	2.5	2.2	1.92
PH	7.9	8.5	8.20	7.75
SAR	5.7	33	24	19.12
Na^+ (meq L^{-1})	7	25	22	17.40
Ca^+ Mg (meq L^{-1})	3	1.04	1.8	1.65
K^+ (meq L^{-1})	0.7	0.5	0.7	1.23
$CO_3{}^{2-}$ (meq L^{-1})	–	–	–	–
$HCO_3{}^-$ (meq L^{-1})	2	15	18	12.0

point (WP) and field capacity (FC) by Pressure Membrane Apparatus. Electrical conductivity of saturation extract, pH, HCO_3, Cl, SO_4 and Ca+Mg were determined according to Jackson [10], organic matter according to Walkley and Black method (as described by Jackson [10]), total nitrogen by the Kjeldahl procedure [10]. Sodium and potassium contents were estimated flame-photometerically, whereas phosphorus

(available) was extracted by $NaHCO_3$ and determined colorimetrically [11]. All the soil characteristics are summarized in Tables 1 and 2. Experiments on each site consisted of three treatments of organic manure (without manure, farmyard manure and sugarcane press mud at 4 t ha^{-1}) in main plots and five treatments of potassium as SOP and MOP (without K, 100, 200 kg K_2O ha^{-1} as SOP and 100 and 200 kg K_2O ha^{-1} as MOP) in subplots with three replications. The seeds of the above-mentioned two sugarcane varieties were obtained from Shakargunj Sugar Mills Ltd, Jhang, Pakistan, and Ayub Agriculture Research Institute Faisalabad, Pakistan. Sowing was done in furrows, and furrow-to-furrow distance was 75 cm at all sites. The sowing was completed during the second and third week of September. The emergence was recorded up to October. Data for plant height was recorded time to time, however, in the present report the data presented on plant height, number of tillers, cane diameter and flag leaf area were recorded during June next year, at all sites. Leaf samples were collected from all four sites, dried and analyzed for Na^+, K^+, Ca^{2+}, P and N.

Soil and water analyses of selected sites for experimentation

The soil analyses (Tab. 1) showed that highest soil salinity was recorded at Jhang (10–20 dS m^{-1}) followed by Samundri (5–17 dS m^{-1}) and NIAB (1–2 dS m^{-1}). The pH of all the soils was more than 7 and SAR at Jhang was again maximum ranging from 27–99 followed by Samundri (29–86) and NIAB (less than one). Na^+ content in the soil of Jhang was higher than Samundri and NIAB, maximum Ca+Mg content was recorded in the soil samples of Jhang (15–20 meq l^{-1}) and minimum at NIAB.

The potassium content of all the soils was almost the same (1–1.56 meq l^{-1}). The carbonate and bicarbonates were absent in the soils of NIAB, while the soil samples of other sites contained HCO_3. The highest HCO_3 were present in soils of Samundri (28–60 meq l^{-1}) followed by Jhang (25–45 meq l^{-1}). The SAR of Jhang was the highest, which affected plant emergence. Soil pH affected nutrient uptake, especially high pH reduced the availability of nutrients. The texture of Jhang was sandy-clay loam while the other two sites, and NIAB and Samundri were clay-loam.

All the sites were irrigated with tube-well water except NIAB where irrigation was done with canal water. The maximum EC of irrigation water was 2.5 dS m^{-1} at the Jhang site having highest SAR (33) followed by Samundri (1.92 dS m^{-1}) with SAR 19 and NIAB (0.77 dS m^{-1}) with SAR 5.7 (Tab. 2). The higher EC and SAR are toxic for plant emergence and growth. Similarly greatest Na contents were observed in tube-well water of Jhang (26 meq L^{-1}) and minimum in that of NIAB (7 meq L^{-1}). The results showed that irrigation water of Jhang is more detrimental than others.

Ca+Mg was highest in the irrigation water of NIAB followed by Samundri and Jhang. High Ca+Mg contents are beneficial for plant growth. Potassium in all irrigation waters is deficient and carbonate is absent in all types of irrigation water used in these experiments. Bicarbonates were recorded in irrigation water and minimum in NIAB. Higher HCO_3 are also toxic for plant growth.

Sugarcane growth and yield

The results showed that salinity reduced sugarcane seed emergence, cane length, diameter and cane yield per plot at all sites under all treatments (Figs. 1 and 2); however, potassium application and amendment with organic manure enhanced seed emergence and all growth and yield parameters. The treatments of SOP or MOP increased growth and yield at all sites with few exceptions. The interactive effect of organic manure and potash showed that SOP performed better than MOP in all organic fertilizers; however, few exceptions were also observed where performance of MOP was better than SOP (Figs. 1 and 2). As the soil salinity in fields was found in patches [12], therefore, the behavior of potash fertilizers was suppressed by high concentrations of salt. However, overall performance of SOP was encouraging in the case of seed emergence, which clearly indicated that SOP was a better fertilizer for sugarcane seed emergence under saline conditions.

The cane yield was calculated on a per hectare basis and the highest yield was obtained from the plants which were growing on normal soil at NIAB followed by saline soil at Samundri, and Jhang (Fig. 1). The application of potash and organic manure significantly enhanced cane yield both under saline and under normal conditions.

Sugarcane variety SPSG-26 performed better than CP-77-400 at NIAB and Samundri while in other places the trend was reversed, which was due to the high salinity in the root media soils selected for SPSG-26. The salt was beyond its tolerant limit, which is why it collapsed. The reduction in cane diameter may be due to the enhancement in cell division and cell division can increase only in those plants which can maintain their turgor potential and from the literature it can be proved that K^+ is helpful in maintaining turgor potential in plants [12]. K^+ is also involved in many metabolic pathways and had major role in increasing plant growth [13].

Nutrient uptake

Na^+ concentration

Na^+ concentration was increased by salinity, lowest Na^+ content was observed in the plants growing under normal conditions at NIAB followed by Samundri and Jhang. Application of organic manures also increased the Na^+ concentration; however, the highest Na^+ concentrations were recorded in those plants that were treated with sugarcane press mud (Fig. 3). The treatments of SOP and MOP reduced the uptake of Na^+ at all sites but the SOP was more effective than MOP. The variety SPSG-26 accumulated less amounts of Na^+ than CP-77-400 at all selected sites.

Potassium

The data clearly indicated that the plants with higher K^+ had higher growth and growth parameters and organic manure amendment and potassium application both increased the K^+ concentration and its availability for plants (Fig. 3). K^+ is involved in maintaining the water status of the plant and turgor potential of its cells. It also has a major role in the opening and closing of stomata. Potassium is also required in the accumulation and translocation of newly prepared carbohydrates [14]. Therefore, the

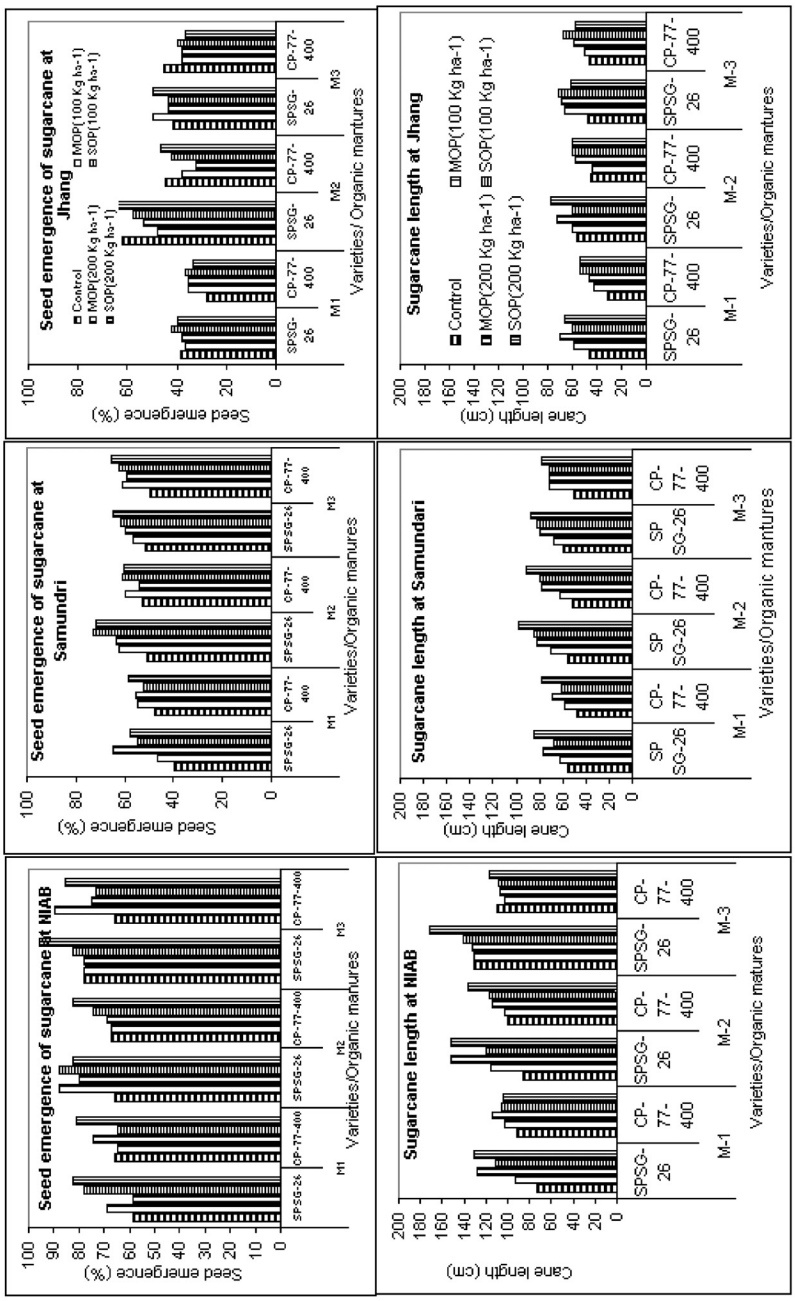

Figure 1. Interactive effect of SOP, MOP and organic manures on sugarcane seed emergence and sugarcane at different selected sites under saline and non-saline conditions

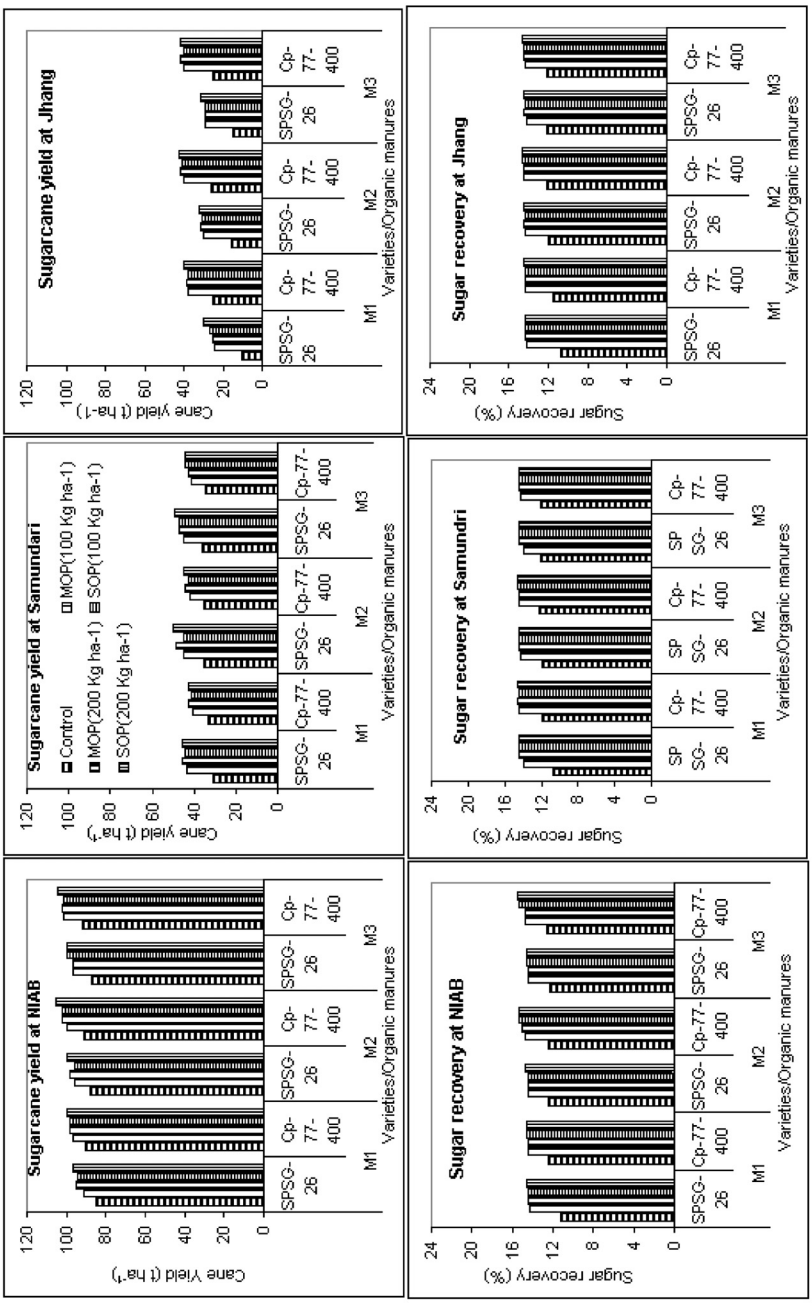

Figure 2. Interactive effect of SOP, MOP and organic manures on sugarcane yield and sugar recovery of sugarcane at different selected sites under saline and non-saline conditions

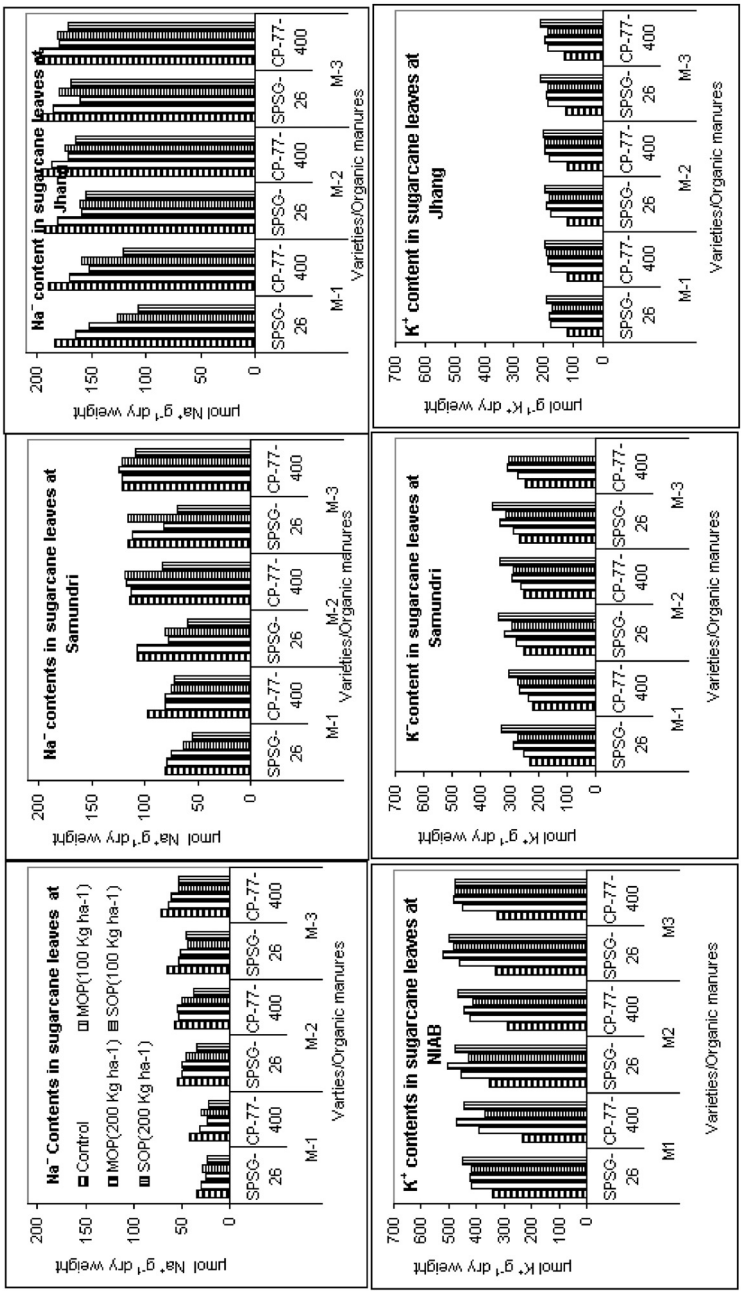

Figure 3. Interactive effect of SOP, MOP and organic manures on Na^+ and K^+ contents in sugarcane leaves at different selected sites under saline and non-saline conditions

better supply of K^+ manages the plant growth up to optimum levels and also maintains plants to adjust osmotically with adverse environments [13]. Salt tolerance is not exclusively correlated with adaptations to Na^+ toxicity *per se* but also reflects adaptations to secondary effects of salinity, such as water deficit and impaired nutrient acquisition. The latter is particularly pertinent where the acquisition of K^+ is concerned due to the physicochemical similarities between Na^+ and K^+ [15]. The capacity of plants to counteract salinity stress will strongly depend on the status of their K^+ nutrition. Conversely, although most plants can cope with external K^+ concentrations, however, physiological 'windows' of optimum K^+ concentrations narrows in the presence of increasing amounts of Na^+ [16].

Calcium

The application of potash as MOP or SOP increased the Ca^{2+} uptake or availability of Ca^{2+} at all selected experimental sites. However, the effect was more pronounced in the SOP treatments (Fig. 4). Ca^{2+} is a major essential element and plays an important role in maintaining cell membrane stability and permeability. It increases germination and growth activities and speeds up the activities of enzymes involved in mitosis, cell division and elongation. It is also important for protein synthesis and carbohydrate transfer, and its presence may serve to detoxify the presence of heavy metals in plants. The availability of Ca^{2+} is necessary for optimum growth and maintenance of metabolic activities in plants growing under stress conditions. Under saline conditions Ca^{2+} compete with Na^+ for common uptake sites and many reports indicate that its uptake is hindered due to salinity [17]. However, the effect of SOP was found to be beneficial in the present study, in increasing either availability or uptake of Ca^{2+}. It is reported that higher Ca:Na ratios promote plant growth, which is very true in the present study as the plants at NIAB and Samundri had higher Ca^{2+} and high growth parameters were recorded there. The K^+ played a major role in the enhancement of Ca^{2+}.

The amendment with organic manures significantly enhanced the Ca^{2+} uptake. Maximum Ca^{2+} concentrations were recorded in the plants treated with sugarcane press mud, closely followed by farmyard manure (FYM). But at NIAB, Ca^{2+} was slightly higher in FYM than that of sugarcane press mud. It is a well established fact that organic manures are not only good sources of organic matter but they also contain some mineral nutrients [6]. So to have a good crop yield, amendment with organic manure is necessary.

Phosphorous

Potash application (SOP and MOP) enhanced the uptake of P; however, the effect of SOP was more pronounced than that of MOP (Fig. 4). The increase in P due to SOP may be due to the growth promoting effect of K and SO_4. Phosphorous is a major essential element necessary for optimum plant growth. It is also a component of certain enzymes and proteins, like adenosine triphosphate (ATP), ribonucleic acids (RNA), DNA and phytin. So its optimum uptake is necessary to maintain growth and metabolism. Its deficiency can affect the DNA and RNA synthesis, which effect not only growth but also genetic signals, required to pass on to seed, and causes

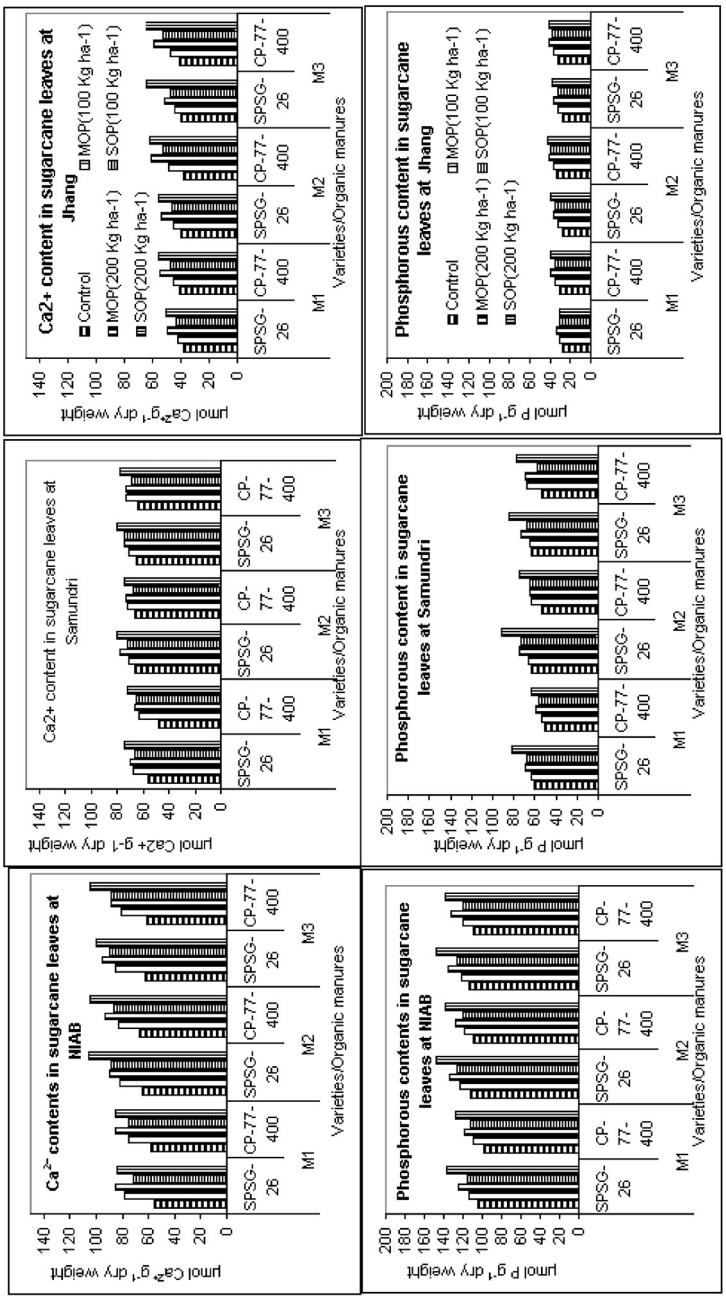

Figure 4. Interactive effect of SOP, MOP and organic manures on Ca^{2+} and phosphorous contents in sugarcane leaves at different selected sites under saline and non-saline conditions

abnormal plant growth in the plants developed from P deficient seeds [18]. The present study revealed that plants with low P had low growth. However, treatment of SOP enhanced P uptake significantly and promoted growth and yield. Amendment with organic manure non-significantly enhanced uptake of P. Both the sources are beneficial in enhancing the P content in soil as well as in plants. From the results it can be concluded that the proper combination of SOP and organic manure can increase the yield of sugarcane and uptake of P.

Nitrogen

Nitrogen concentration reduced with salinity. Highest N contents were recorded in plants growing under normal conditions, followed by Samundri and Jhang (Fig. 5). Although salinity at Samundri was maximum at the time of sowing, the constant use of gypsum with water reduced it, and plant growth and nutrient uptake was better there than all other selected sites. It is well documented that salinity reduces the uptake of N; however, application of K enhances N in plants. Salinity may reduce the synthesis of certain enzymes involved in the nitrogen metabolism, such as nitrate reductase, or may reduce the substrate due to the presence of high salts in the root medium.

The data clearly indicated that the application of K significantly increased the uptake of nitrogen. Nitrogen uptake was enhanced by both sources, i.e., SOP and MOP; however, SOP was more efficient in increasing nitrogen concentrations than that of MOP. It is well known that nitrogen and potassium are plant nutrients required in high amounts for good crop growth and high production. Nitrogen is an essential element of biomolecules such as amino acids, proteins, nucleic acids, phytohormones and a number of enzymes and coenzymes. Nitrogen is deeply involved in the first step of growth which means the replication of chromosomes and which in biochemical terms consists mainly of the synthesis of deoxyribonucleic acids and nuclear protein [19]. On the other hand, potassium has no particular function as a constitutive element of biomolecules. Nevertheless, it is also an essential element for plants. Numerous physiological processes are known in which K^+ is involved and it was found that nitrogen and K^+ influence plant growth in a synergistic way. K^+ uptake into cells may contribute to the osmotic potential of the cytoplasm, which is a requisite of the osmotic water uptake. It is in the literature that K^+ has an activating effect on ATPase. It also contributes to depolarize the membrane by virtue of which ATPase activity increases [20, 21]. The permanent supply of K^+ to meristematic tissues stimulates the growth processes. This is why the plant treated with K^+ had better growth. In the growth process, phytohormones and K^+ are involved in a synergistic way. Meristematic growth is the prerequisite of crop production. It is for this reason that a balanced K^+ and N supply is of such a high relevance for having high yield.

The results about varietal performance showed that SPSG-26 took up N at NIAB and Samundri more efficiently than Jhang, where CP-77-400 showed better performance. Although SPSG-26 is salt tolerant (up to 10 dSm^{-1}), the plots selected for this variety had very high salinity and no additional gypsum treatment was imposed at these two places due to which SPSG-26 did not perform well.

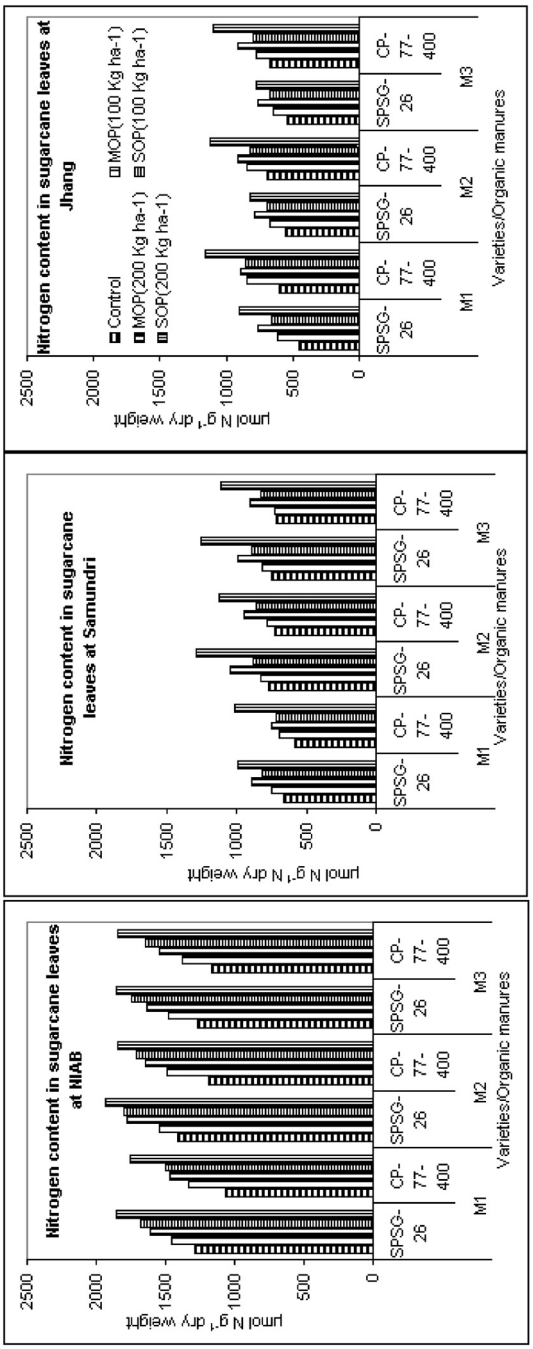

Figure 5. Interactive effect of SOP, MOP and organic manures on nitrogen contents in sugarcane leaves at different selected sites under saline and non-saline conditions

The amendment of organic manures also increased the uptake of nitrogen in both the varieties. The farmyard manure showed pronounced effects on nitrogen uptake. The organic manures are good sources of nitrogen and the importance of nitrogen in growth and other metabolic processes have been discussed above. From these results it can be concluded that for better plant growth, high yield and high nutrient availability to plants, a suitable combination of organic manure, nitrogen and potassium is required.

Conclusion

From the results of the present study, it can be concluded that for optimum sugarcane growth and yield, balanced amount of K^+ and N in combination with organic manures are required. The fertilizer rates of this nutrient should be 50 % more for saline soil as compared to normal ones. For optimum sugarcane yield, the salinity of the soil selected for sugarcane cultivation should not be more than 10 dS m^{-1}, otherwise even tolerant varieties fail to produce economical yields. Selection of salt tolerant varieties is also of great importance in order to achieve higher sugarcane yields. SOP is more beneficial than MOP in many ways as has been discussed in this chapter.

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Biosaline Agriculture and Salinity Tolerance in Plants
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The effects of saline irrigation water by drip irrigation on salt distribution in soil

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Introduction

Depending on the increase in the salt amount in irrigation water in many arid and semi-arid regions of the world, salinity problems in agricultural soils appears and agricultural production is inhibited by excessive salt concentrations. This type of salinity occurs in topographical lowlands near the sea where intrusion of seawater to the aquifer is inevitable [1]. The reason why salinity is high in the areas near the sea, or in areas gained from the sea, is due to seawater intrusion. The salt composition of these soils is the same with that of the seawater [2]. The salinity of the seawater is accepted as approximately 0.35 %. The concentrations of main ions in seawater are 19.35 me L^{-1} Cl^{-}, 10.752 me L^{-1} Na^{+}, 2.701 me L^{-1} $SO_4^{=}$, 1.295 me L^{-1} Mg^{+2} and 0.39 me $L^{-1}K^{+}$ [3]. Approximately 70 % of the world's population lives near, or at, the seaside. The rise in the population and the settlement in these areas increase the agricultural and industrial activities, hence a pressure on water sources takes place. The greatest effect of this pressure is on the change in the quality of underground water sources [4]. Various factors affect the salinity of underground water sources. The most important reason for the salinity in the aquifers at the seaside is the seawater intrusion [5].

Satsuma mandarin, which generally grow in the Gümüldür district of Izmir, Turkey, is economically important for this region. However in recent years, because of the seawater intrusion to the underground water sources, salinity problems in these soils have been of concern [6].

In the summer months, when mandarin orchards need irrigation, domestic water consumption increases also due to the rise in tourism. Further, seawater enters in the discharged underground water sources. For this reason, salinity stress in this region has serious effects on plant growth in the months of August and September.

The objective of the present study was to investigate the distribution of salinity using saline water with drip irrigation.

Material and methods

Soil samples were taken from an experimental site which had been established in 1996 to determine the effects of salinity on yield and quality of Satsuma mandarins (cv. Owari) budded onto *Poncirus trifoliata* and *Troyer citrange* rootstocks at Ege University Campus in Izmir, Turkey. The tree spacing was 3 m between rows and 2.5 m on rows. Five different levels of irrigation with saline water (0.65 (fresh water-I_0), 2.00 (I_1); 3.50 (I_2); 5.00 (I_3) and 6.50 (I_4) dS m^{-1}) was realized during the summer months of 1996–2000 by drip irrigation [6]. Treatment plots were randomly located in each of four replicate blocks. Recommended amounts of nitrogen (N), phosphorus (P) and potassium (K) were applied. Soil samples were taken from the experimental field two times, on 16 August 2000 and 31 October 2000. Although the effective precipitation in the region up to the first sampling time did not occur, in October (which was the second sampling time), an average of 63 mm precipitation fell.

The drips with 2.3 Lh^{-1} flow rate had the pressure regulated system and online type. Each different salt level contains different subjects (I_0, I_1, I_2, I_3 and I_4) and each subject contains three trees. There are a total of 240 mandarin trees in this experiment. The amounts of water and salt applied between the years 1996–2000 are given in Table 1, and the precipitation in Table 2. Soil samples were taken from two different depths (0–20 cm and 20–40 cm), and three different distances from the plant stem (0–20, 20–40 and 40–60 cm) in order to study clearly the salt distribution in the soil. The sampling was made for between (BR) and on rows (OR). Some physical and chemical properties of the experimental soil are given in Table 3 [6]. Soil samples were air dried and sieved (2 mm) before analyses [7]. Soil texture was determined by hydrometer method [8] and soil reaction and conductivity were measured in a 1:2.5 (W/W) aqueous solution [9]. Total $CaCO_3$ was assessed by using Scheibler calcimeter [10]. Furthermore, in the saturation extracts of soils pH [9], Na [9] and Cl [7] were determined. TARIS program was used in the statistic analysis of all data.

Table 1. The amounts of water and salt applied between 1996 and 2000

Years	Beginning and Ending Date of Irrigation	Total Applied Water		Total Applied Salt (kg)
		(mm)	(liter)	
1996	31 May – 15 November	695.30	166,872	–
1997	27 May – 23 October	517.36	124,166	163.50
1998	18 June – 12 October	521.19	125,086	160.00
1999	11 June – 12 November	443.52	106,445	158.00
2000	01 June – 14 November	467.22	112,132	166.52

Table 2. Precipitation between 1996 and 2000 (mm)

Years	1996	1997	1998	1999	2000
Total Year	548.50	616.20	839.40	620.60	530.20
Average Month	45.71	51.35	69.95	51.72	44.18

Table 3. Some physical and chemical properties of the experiment soil in 1996

pH (H_2O)	Total $CaCO_3$ (%)	EC_e ($\mu S\ cm^{-1}$)	Sand (%)	Silt (%)	Clay (%)	Texture
7.35	1.16	550	66.88	19.84	13.28	Sandy loam

Results and discussion

Some chemical properties of the soils taken from 0–30 cm depth, and from 30 cm distance from the plant stem as control treatment in the first period are given in Table 4. The results that belong to the control parcel are accepted as the same for both *Poncirus trifoliata* and *Troyer citrange* rootstocks.

According to these results, the pH of the control parcel was analyzed as 5.84 and 4.57 in the direction of between and on rows, respectively. Total soluble salts in the water (%) did not change in relation to directions and was determined as 0.201 % on average. The electrical conductivities of the saturation extracts were found to be 3,550 $\mu S\ cm^{-1}$ and 3,650 $\mu S\ cm^{-1}$ between and on rows, respectively. The saturation extract of the control treatment contained an average of 2.95 me l^{-1} Na^+ and 15.48 me l^{-1} Cl^-.

Results related to the first period showed a linear increase in electrical conductivity of the saturation extract (EC_e) as well as the amount of soluble salts (%) at 0–20 cm soil depth depending upon the amount of added salt (Figs. 1a, 1b, 2a, 2b).

The amounts of soluble salts and EC_e values on rows were higher than that of between rows because of the more salt accumulation on the laterals between drips. In addition, according to the results obtained from both rootstocks, the EC_e values in both depths decreased as the distance from the plant stem increased (Tabs. 4 and 5; Figs. 1a, 1b).

Parallel to the increase in the salinity, the increases were also observed in the concentration of Na^+ and Cl^- ions in the soil saturation extract. Na^+ concentrations of soils taken from 0–20 cm depth and from on rows of *Poncirus trifoliata* were determined as 16.96, 25.99, 29.98 and 31.75 me l^{-1} depending on the enhanced salt rates (Figs. 3a and 3b). Similarly, Cl^- concentrations were found to be 31.96, 34.94, 44.97 and 52.94 me l^{-1} (Tab. 4; Figs. 4a and 4b).

In *Troyer citrange* rootstock these values were found to be 23.21, 24.93, 30.97 and 35.23 me l^{-1} for Na^+ and 31.96, 36.96, 46.94 and 54.97 me l^{-1} for Cl^- (Tab. 5; Figs. 3a, 3b, 4a, 4b). In the saturation extract of the control soil, the Na^+ and Cl^- concentration were 2.83 and 15.00 me l^{-1} on rows and 3.06 and 15.96 me l^{-1} between rows, respectively. The reason for the difference between Na^+ and Cl^- concentrations of both rootstocks at the same treatment resulted from the different

Table 4. Chemical properties of *Poncirus trifoliata* soils (first sampling period)

Treatment	Direction*	Depth (cm)	Distance (cm)	Total Soluble Salt (%)	pH (1:2.5)	Saturation extract			
						pH_e	EC_e $\mu S\,cm^{-1}$	me l^{-1} Na^+	me l^{-1} Cl^-
Control	OR	30	0–30	0.200	5.84	6.47	3,550	2.83	15.00
	BR	30	0–30	0.202	4.57	6.59	3,650	3.06	15.96
I_1(2.0 dS m^{-1})	BR	20	0–20	0.245	6.32	6.85	4,800	17.00	30.90
	BR	20	20–40	0.205	5.15	5.70	3,710	10.20	26.96
	BR	20	40–60	0.204	7.37	6.76	3,640	5.87	25.97
	BR	40	0– 20	0.212	7.16	6.69	3,365	11.31	26.98
	BR	40	20–40	0.195	7.35	6.63	3,075	10.44	22.97
	BR	40	40–60	0.200	7.36	6.68	3,442	8.48	20.86
	OR	20	0–20	0.249	6.99	6.83	5,010	16.96	31.96
	OR	20	20–40	0.220	7.37	6.71	4,260	13.27	28.96
	OR	20	40–60	0.188	7.46	6.83	2,880	6.31	20.97
	OR	40	0–20	0.200	7.08	6.81	2,910	8.87	20.98
	OR	40	20–40	0.203	7.29	6.88	3,702	11.31	25.97
	OR	40	40–60	0.165	7.45	6.84	2,910	6.52	19.97
I_2(3.5 dS m^{-1})	BR	20	0–20	0.350	5.70	6.52	5,940	25.10	36.95
	BR	20	200–40	0.298	6.98	6.85	4,800	17.02	31.96
	BR	20	40–60	0.180	7.30	6.91	2,830	4.39	20.98
	BR	40	0–20	0.313	6.37	7.01	5,210	18.93	35.93
	BR	40	20–40	0.300	7.04	6.66	4,650	12.88	28.96
	BR	40	40–60	0.205	4.84	7.17	3,300	5.00	18.96
	OR	20	0–20	0.350	7.35	5.96	5,990	25.99	34.94
	OR	20	20–40	0.320	6.66	6.85	5,450	18.36	33.95
	OR	20	40–60	0.211	7.28	6.79	4,070	9.57	26.97
	OR	40	0–20	0.285	6.69	6.89	5,100	21.10	32.94
	OR	40	20–40	0.237	6.92	6.67	3,810	11.74	26.96
	OR	40	40–60	0.237	7.21	6.64	3,790	8.26	25.97
I_3(5.0 dS m^{-1})	BR	20	0–20	0.380	4.85	6.20	6,950	28.26	44.93
	BR	20	20–40	0.310	6.85	6.88	5,180	20.23	34.95
	BR	20	40–60	0.202	7.30	7.23	3,445	11.53	22.97
	BR	40	0–20	0.330	6.13	6.91	5,350	25.66	36.95
	BR	40	20–40	0.245	7.11	7.40	4,400	16.22	33.97
	BR	40	40–60	0.210	7.42	6.92	3,382	8.70	21.96
	OR	20	0–20	0.385	4.81	6.19	7,190	29.98	44.97
	OR	20	20–40	0.320	5.23	6.29	5,620	25.23	37.96
	OR	20	40–60	0.238	6.94	6.76	4,180	14.35	28.96
	OR	40	0–20	0.345	6.14	6.56	5,880	23.27	39.96
	OR	40	20–40	0.290	6.84	6.98	4,850	19.31	28.96
	OR	40	40–60	0.215	7.40	7.03	3,560	12.40	21.97
I_4(6.5 dS m^{-1})	BR	20	0–20	0.400	6.77	6.82	7,400	31.75	51.93
	BR	20	20–40	0.310	7.34	6.78	5,580	25.75	41.96
	BR	20	40–60	0.220	7.37	7.02	4,010	10.74	30.95
	BR	40	0–20	0.318	7.35	6.97	5,450	23.79	40.94
	BR	40	20–40	0.260	7.47	6.98	4,120	16.09	30.95
	BR	40	40–60	0.225	7.43	6.97	3,541	7.61	27.00
	OR	20	0–20	0.410	6.51	7.37	7,520	31.75	52.94
	OR	20	20–40	0.362	7.02	7.06	6,110	27.40	42.94
	OR	20	40–60	0.242	7.45	7.32	3,840	9.53	31.96
	OR	40	0–20	0.365	7.23	7.16	5,940	25.23	39.97
	OR	40	20–40	0.308	7.30	7.49	5,065	21.31	35.95
	OR	40	40–60	0.220	7.50	7.5	3,440	8.15	25.83

** **BR:** Between rows direction * **OR:** On rows direction*

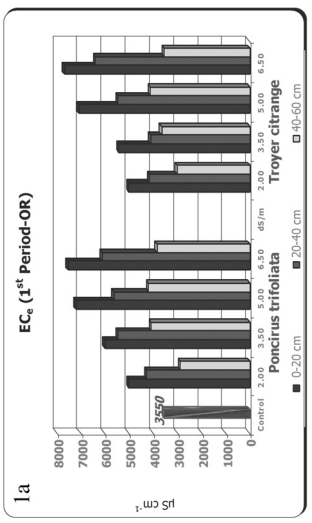

Figure 1a. EC$_e$ values at 0–20 cm depth of soils taken on rows (OR) in the first period

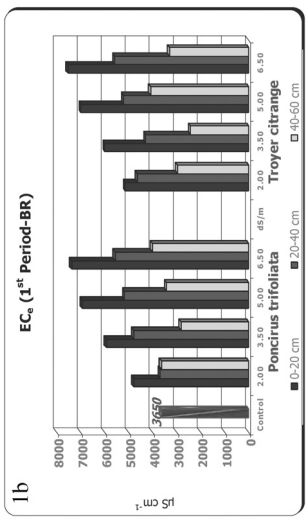

Figure 1b. EC$_e$ values at 0–20 cm depth of soils taken between rows (BR) in the first period

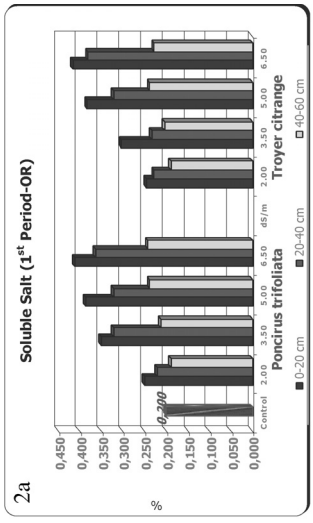

Figure 2a. Amount of total soluble salt at 0–20 cm depth of soils taken on rows (OR) in the first period

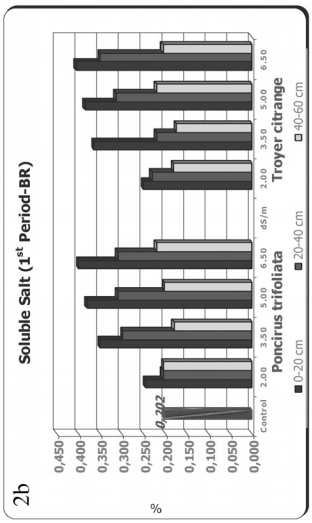

Figure 2b. Amount of total soluble salt at 0–20 cm depth of soils taken between rows (BR) in the first period

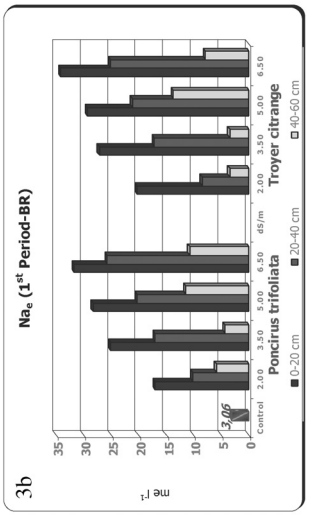

Figure 3a. Na_e^+ values at 0–20 cm depth of soils taken on rows (OR) in the first period

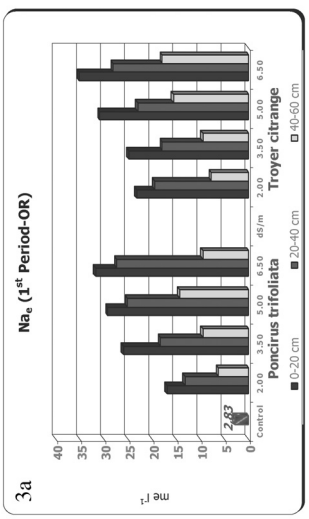

Figure 4a. Cl_e^- values at 0–20 cm depth of soils taken on rows (OR) in the first period

Figure 3b. Na_e^+ values at 0–20 cm depth of soils taken between rows (BR) in the first period

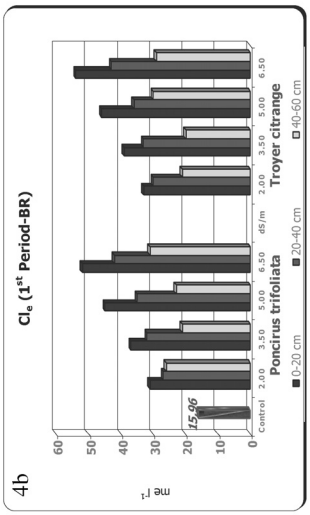

Figure 4b. Cl_e^- values at 0–20 cm depth of soils taken between rows (BR) in the first period

characteristic of the rootstocks [6, 11]. The increase in the Na_e and Cl_e in relation to increased salinity at the first depth and distance also occurred at the second distance. But this significant increase could not be determined at the farthest distance (40–60 cm) from the plant stem in each salt treatment (Figs 3a, 3b, 4a, 4b).

In the soils of the second period, salinity increased as the distance of drippers and the soil depth increased. Nevertheless, in these soils where more saline irrigation water was applied, the amount of total soluble salt, EC_e, Na_e^+ and Cl_e^- decreased in the soil under the soil surface (Tabs. 6 and 7). The increase of these parameters depending on the increased soil depth showed that the leaching occurred under the surface. It can be said that an average of 63 mm of rain fell during the second sampling period, and from October leached the salts to deeper zones and carried throughout the laterals.

Na_e and EC_e values at 0–20 cm soil depth, just under the drippers significantly decreased compared to the first period. However, these parameters clearly increased at the distances of 0–20 cm and 20–40 cm from the stem in both directions (Figs. 5a, 5b, 7a, 7b). The characteristic leaching area occurred just under the drippers caused by the moving of salts from this region and accumulating in the outside of the leaching area throughout laterals (Figs. 6a and 6b).

In the second period soils, the concentration of Cl^- ion increased in all directions (OR and BR) and distances (0–20, 20–40 and 40–60 cm) for all salt treatments except for I_1 treatment between rows at the first distance compared to the first period (Figs. 8a and 8b).

The fact that Na^+ and Cl^- could not leach deeper soil layers and ions move to upper layers by capillarity in arid conditions, the concentrations of Na^+ and Cl^- decreased at 20–40 cm soil depth of the first period. Whereas in the soils sampled in the second period, higher amounts of saline water were applied, the leaching effect of rain occurred and water movement became slow by capillarity. For this reason, the leaching of salts from the soil surface occurred in higher levels than the first period soils and the concentrations of Na^+ and Cl^- at the second depth clearly increased in both rootstocks compared to the first period.

Irrigating the soils with more saline water in the second period, less salt under the drips which is a characteristic of drip irrigation systems and 63 mm rainfall in October 2000 affected the distribution of $Cl^-$and Na^+ ions, as considered above.

Similarly to Na^+ ion, Cl^- ion also increased between and on rows of two of the rootstocks.

The decrease of soil reaction in the second period could have resulted from HNO_3 used for adjusting the pH of nutrient solution and for preventing plugging of laterals and drippers used in the irrigation system. While the pH of the first period soils varied from 4.50–7.52, in the second period these values were in the range of 5.12–7.22.

In the first period samples, while salt accumulation occurred at 0–20 cm soil depth and 0–20 cm distance from the stem between and on rows, in the second period samples, salt moved to 20–40 and 40–60 cm distances from the stem and to 20–40 cm soil depth. For this reason, salt accumulation occurred at a greater distance from the drippers. In fact, salt accumulates outside the wetting area in drip irrigation.

Table 5. Chemical properties of *Troyer citrange* soils (first sampling period)

Treatment	Direction*	Depth (cm)	Distance (cm)	Total Soluble Salt (%)	pH (1:2.5)	Saturation extract			
						pH_e	EC_e $\mu S\,cm^{-1}$	me l^{-1} Na^+	me l^{-1} Cl^-
I_1 (2.0 dS m^{-1})	BR	20	0–20	0.250	4.50	6.36	5,150	20.24	32.95
	BR	20	20–40	0.230	6.78	7.24	4,654	8.51	29.97
	BR	20	40–60	0.181	7.18	7.51	2,980	3.48	20.98
	BR	40	0–20	0.210	6.71	7.40	3,950	12.96	27.95
	BR	40	20–40	0.188	7.15	7.35	3,150	7.16	23.83
	BR	40	40–60	0.175	7.10	7.27	3,060	6.06	19.98
	OR	20	0–20	0.245	4.55	5.81	5,000	23.21	31.96
	OR	20	20–40	0.227	5.74	7.14	4,150	19.54	29.96
	OR	20	40–60	0.188	7.34	7.57	3,010	7.74	22.97
	OR	40	0–20	0.232	6.71	7.45	3,750	10.92	27.95
	OR	40	20–40	0.230	7.10	7.40	3,640	8.64	26.95
	OR	40	40–60	0.203	7.39	7.33	3,260	7.16	20.97
I_2 (3.5 dS m^{-1})	BR	20	0–20	0.365	5.14	6.25	6,010	27.12	38.96
	BR	20	20–40	0.220	6.81	7.40	4,300	17.21	32.97
	BR	20	40–60	0.175	7.36	7.59	2,450	3.48	19.96
	BR	40	0–20	0.243	6.66	7.30	4,760	17.99	33.86
	BR	40	20–40	0.232	7.00	7.27	4,550	19.54	25.83
	BR	40	40–60	0.162	7.43	7.58	2,645	8.51	20.98
	OR	20	0–20	0.303	4.99	6.51	5,420	24.93	36.96
	OR	20	20–40	0.232	6.03	6.80	4,090	17.96	26.97
	OR	20	40–60	0.203	7.15	6.67	3,650	9.53	23.98
	OR	40	0–20	0.260	6.39	6.79	4,930	21.29	29.89
	OR	40	20–40	0.203	6.87	7.00	3,340	17.23	23.96
	OR	40	40–60	0.170	7.40	7.17	2,640	9.18	20.98
I_3 (5.0 dS m^{-1})	BR	20	0–20	0.385	5.39	6.55	7,010	29.36	45.94
	BR	20	20–40	0.314	6.69	7.52	5,220	21.18	35.87
	BR	20	40–60	0.220	7.34	7.66	4,100	13.80	29.98
	BR	40	0–20	0.358	6.65	7.26	6,010	27.40	40.96
	BR	40	20–40	0.290	6.98	7.23	5,120	20.66	32.97
	BR	40	40–60	0.220	7.19	7.23	4,050	11.70	26.96
	OR	20	0–20	0.382	5.18	6.35	7,075	30.97	46.94
	OR	20	20–40	0.320	7.04	7.27	5,450	23.05	40.97
	OR	20	40–60	0.238	7.29	7.32	4,110	15.66	30.97
	OR	40	0–20	0.350	6.13	7.11	5,910	29.58	42.94
	OR	40	20–40	0.280	6.97	7.32	4,655	22.47	32.94
	OR	40	40–60	0.218	7.17	7.30	4,000	14.15	27.97
I_4 (6.5 dS m^{-1})	BR	20	20–40	0.350	7.08	6.94	5,590	25.10	42.94
	BR	20	40–60	0.205	7.48	7.09	3,310	7.96	28.97
	BR	40	0–20	0.374	6.83	7.19	6,180	32.59	42.96
	BR	40	20–40	0.354	7.20	7.10	5,640	29.80	36.95
	BR	40	40–60	0.272	7.42	7.21	4,120	20.16	27.94
	OR	20	0–20	0.415	7.10	6.71	7,670	35.23	54.97
	OR	20	20–40	0.380	6.90	7.06	6,380	28.20	43.97
	OR	20	40–60	0.227	7.52	7.19	3,550	17.96	27.96
	OR	40	0–20	0.377	6.53	7.02	6,170	27.09	43.94
	OR	40	20–40	0.342	7.06	7.00	5,642	24.65	37.95
	OR	40	40–60	0.280	7.48	7.16	4,370	18.56	31.94

** **BR:** Between rows direction * **OR:** On rows direction*

Table 6. Chemical properties of *Poncirus trifoliata* soils (second sampling period)

Treatment	Direction*	Depth (cm)	Distance (cm)	Total Soluble Salt (%)	pH (1:2.5)	Saturation extract			
						pH_e	EC_e $\mu S\,cm^{-1}$	me l^{-1} Na^+	me l^{-1} Cl^-
Control	OR	30	0–30	0.200	6.97	7.27	3,250	4.40	15.83
	BR	30	0–30	0.205	6.02	6.88	3,400	3.67	14.96
I_1 (2.0 dS m^{-1})	BR	20	0–220	0.281	6.67	7.55	4,320	12.73	26.97
	BR	20	20–40	0.320	7.01	7.40	5,010	13.97	29.97
	BR	20	40–60	0.334	7.15	7.49	5,210	18.51	31.97
	BR	40	0–20	0.180	7.08	7.68	3,105	12.32	21.97
	BR	40	20–40	0.281	7.04	7.69	4,440	13.82	24.97
	BR	40	40–60	0.294	6.71	7.59	4,864	15.97	28.97
	OR	20	0–20	0.276	6.17	7.39	4,150	13.97	28.96
	OR	20	20–40	0.348	7.15	7.54	5,123	15.62	29.96
	OR	20	40–60	0.348	7.21	7.48	5,390	16.27	31.97
	OR	40	0–20	0.180	6.57	7.65	3,056	11.67	22.96
	OR	40	20–40	0.262	7.06	7.63	4,290	11.02	23.97
	OR	40	40–60	0.330	7.22	7.56	5,390	18.60	29.96
I_2 (3.5 dS m^{-1})	BR	20	20–40	0.392	6.59	7.03	6,840	24.78	44.96
	BR	20	40–60	0.349	6.73	7.12	5,520	22.41	40.91
	BR	40	0–20	0.335	6.52	7.01	5,285	21.49	35.92
	BR	40	20–40	0.389	6.60	6.82	6,245	23.04	42.92
	BR	40	40–60	0.401	6.39	7.09	6,854	25.18	45.93
	OR	20	0–20	0.300	6.53	6.84	4,670	20.19	34.91
	OR	20	20–40	0.349	6.46	6.96	5,640	21.63	37.96
	OR	20	40–60	0.383	6.82	7.23	6,240	23.20	44.96
	OR	40	0–20	0.307	6.18	7.16	4,820	21.20	35.92
	OR	40	20–40	0.392	5.79	7.30	6,100	24.10	42.87
	OR	40	40–60	0.360	6.68	6.96	5,780	22.20	44.83
I_3 (5.0 dS m^{-1})	BR	20	0–20	0.381	5.12	6.36	6,340	25.79	46.91
	BR	20	20–40	0.381	5.54	6.72	6,800	26.15	49.92
	BR	20	40–60	0.368	5.76	6.60	6,350	23.20	43.96
	BR	40	0–20	0.393	5.18	6.12	7,010	28.10	51.97
	BR	40	20–40	0.393	5.63	6.77	7,050	28.56	52.93
	BR	40	40–60	0.406	5.87	6.98	7,200	30.04	55.92
	OR	20	0–20	0.361	6.05	7.29	6,250	23.18	44.97
	OR	20	20–40	0.357	5.94	7.22	6,105	26.42	48.96
	OR	20	40–60	0.387	5.92	7.00	6,802	29.18	56.97
	OR	40	0–20	0.372	5.65	6.90	6,640	26.20	48.92
	OR	40	20–40	0.400	6.29	6.75	7,215	30.64	56.91
	OR	40	40–60	0.387	6.46	6.82	6,980	29.00	55.91
I_4 (6.5 dS m^{-1})	BR	20	0–20	0.440	7.14	7.45	7,120	29.18	55.83
	BR	20	20–40	0.465	7.06	7.36	7,460	30.41	59.91
	BR	20	40–60	0.465	7.07	7.53	7,500	32.13	63.87
	BR	40	0–20	0.431	7.16	7.50	7,010	27.28	52.94
	BR	40	20–40	0.473	7.10	7.50	7,650	33.51	64.92
	BR	40	40–60	0.479	7.14	7.62	7,715	37.20	68.81
	OR	20	0–20	0.420	6.89	7.39	7,010	27.25	52.87
	OR	20	20–40	0.423	6.95	7.45	7,195	29.64	57.91
	OR	20	40–60	0.430	6.92	7.60	7,220	28.80	55.93
	OR	40	0–20	0.429	7.00	7.56	7,073	27.43	53.81
	OR	40	20–40	0.460	6.92	7.68	7,495	34.01	62.93

** **BR:** Between rows direction * **OR:** On rows direction*

Table 7. Chemical properties of *Troyer citrange* soils (second sampling period)

Treatment	Direction*	Depth (cm)	Distance (cm)	Total Soluble Salt (%)	pH (1:2.5)	Saturation extract			
						pH_e	EC_e $\mu S\,cm^{-1}$	me l^{-1} Na^+	me l^{-1} Cl^-
I_1 (2.0 dS m^{-1})	BR	20	0–20	0.260	6.79	7.65	4,500	18.98	28.93
	BR	20	20–40	0.275	6.83	7.68	4,430	15.20	28.94
	BR	20	40–60	0.293	6.90	7.70	4,850	19.30	32.94
	BR	40	0–20	0.190	6.80	7.52	3,450	15.73	26.96
	BR	40	20–40	0.263	6.73	7.50	4,220	16.70	27.94
	BR	40	40–60	0.297	6.92	7.65	4,860	18.10	30.97
	OR	20	0–20	0.210	6.29	7.29	3,790	16.53	25.91
	OR	20	20–40	0.290	6.46	7.35	4,680	18.25	29.83
	OR	20	40–60	0.290	6.37	7.38	4,795	19.10	30.96
	OR	40	0–20	0.200	6.37	7.30	3,550	16.23	25.93
	OR	40	20–40	0.270	6.52	7.62	4,320	17.43	29.94
	OR	40	40–60	0.300	6.40	7.52	4,886	18.24	32.96
I_2 (3.5 dS m^{-1})	BR	20	0–20	0.365	6.52	7.49	6,053	19.20	39.83
	BR	20	20–40	0.360	6.41	7.42	5,920	20.49	40.83
	BR	20	40–60	0.323	6.37	7.63	5,460	21.86	41.89
	BR	40	0–20	0.371	6.29	7.56	6,110	21.18	42.97
	BR	40	20–40	0.374	6.46	7.60	6,250	22.10	44.96
	BR	40	40–60	0.390	6.39	7.60	6,362	23.20	45.87
	OR	20	0–20	0.303	6.54	7.40	4,750	20.38	34.96
	OR	20	20–40	0.335	6.29	7.53	5,696	24.03	36.93
	OR	20	40–60	0.370	6.70	7.46	6,256	25.20	42.97
	OR	40	0–20	0.300	6.49	7.39	5,069	24.32	38.96
	OR	40	20–40	0.372	6.84	7.56	6,272	27.20	44.97
	OR	40	40–60	0.349	6.60	7.40	5,897	26.18	41.96
I_3 (5.0 dS m^{-1})	BR	20	0–20	0.374	5.90	7.26	6,725	26.96	48.91
	BR	20	20–40	0.379	6.05	7.00	6,805	28.19	51.92
	BR	20	40–60	0.381	6.20	7.10	6,890	24.49	50.92
	BR	40	0–20	0.385	6.00	7.22	6,985	29.38	53.92
	BR	40	20–40	0.390	6.10	7.26	7,010	28.90	56.91
	BR	40	40–60	0.398	6.19	7.29	7,150	31.18	57.91
	OR	20	0–20	0.352	6.25	7.30	6,500	25.94	46.92
	OR	20	20–40	0.370	6.30	7.51	6,653	26.95	51.92
	OR	20	40–60	0.390	6.25	7.46	6,948	29.38	54.92
	OR	40	0–20	0.370	6.32	7.53	6,648	27.40	49.92
	OR	40	20–40	0.405	6.29	7.51	7,250	29.90	60.92
	OR	40	40–60	0.400	6.34	7.60	7,175	30.05	57.92
I_4 (6.5 dS m^{-1})	BR	20	0–20	0.420	6.35	7.58	7,000	29.96	53.96
	BR	20	20–40	0.440	6.56	7.45	7,310	30.86	57.96
	BR	20	40–60	0.469	6.27	7.60	7,553	34.91	68.96
	BR	40	0–20	0.435	6.35	7.34	7,115	29.44	54.93
	BR	40	20–40	0.490	6.46	7.39	7,785	36.19	68.83
	BR	40	40–60	0.450	6.50	7.50	7,480	37.30	66.87
	OR	20	0–20	0.400	6.43	7.28	7,015	27.86	54.97
	OR	20	20–40	0.427	6.60	7.37	7,151	29.44	58.81
	OR	20	40–60	0.443	6.29	7.20	7,511	36.20	61.86
	OR	40	0–20	0.417	6.37	7.35	7,108	28.88	55.87
	OR	40	20–40	0.468	6.50	7.45	7,550	37.64	64.96
	OR	40	40–60	0.441	6.50	7.00	7,384	32.47	62.96

** **BR:** Between rows direction * **OR:** On rows direction*

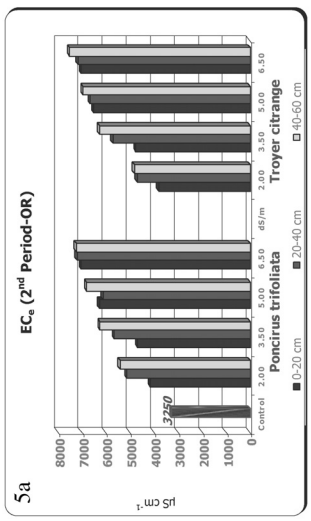

Figure 5a. EC_e values at 0–20 cm depth of soils taken on rows (OR) in the second period

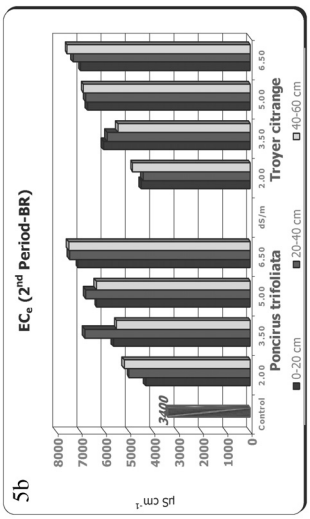

Figure 5b. EC_e values at 0–20 cm depth of soils taken between rows (BR) in the second period

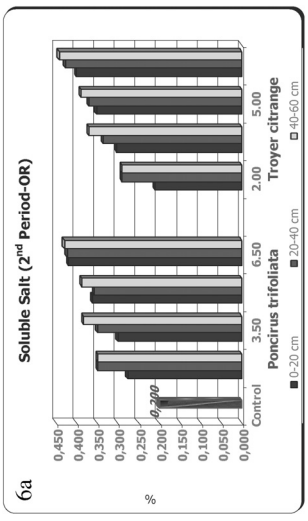

Figure 6a. Amount of total soluble salt at 0–20 cm depth of soils taken on rows (OR) in the second period

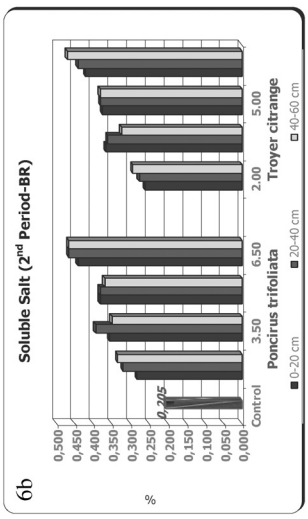

Figure 6b. Amount of total soluble salt at 0–20 cm depth of soils taken between rows (BR) in the second period

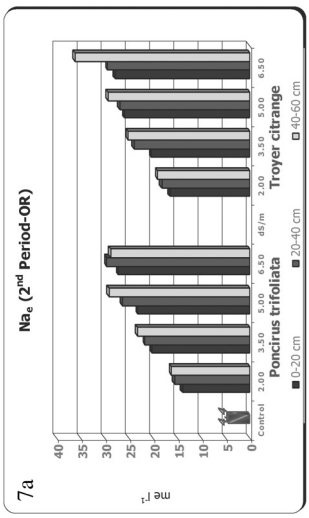

Figure 7a. Na_e^+ values at 0–20 cm depth of soils taken on rows (OR) in the second period

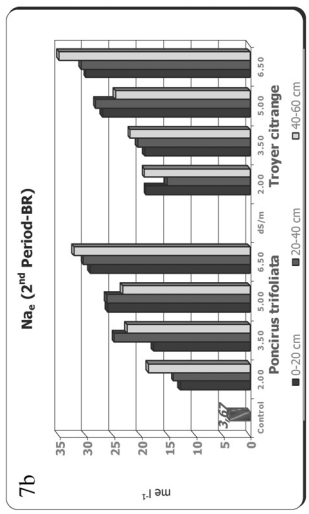

Figure 7b. Na_e^+ values at 0–20 cm depth of soils taken between rows (BR) in the second period

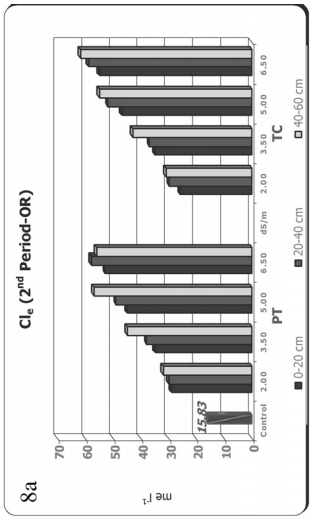

Figure 8a. Cl_e^- values at 0–20 cm depth of soils taken on rows (OR) in the second period

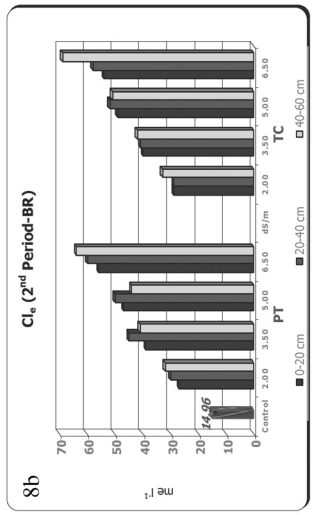

Figure 8b. Cl_e^- values at 0–20 cm depth of soils taken between rows (BR) in the second period

The reason for the deeper leaching of salts was probably due to the 63 mm rainfall during October.

According to the variance analysis and LSD test, a 1 % significant relationship was found between the applied salt and total soluble salts, electrical conductivity of saturation extract (EC_e), Na^+ and Cl^- in this extract.

The fact that the composition of salt applied to soils is the same with that of seawater (dominant salt NaCl), only the concentration of Na^+ and Cl^- showed a linear increase depending on the increased salt levels.

Soil reaction depending on the salt levels was negative at 1 % significance level in the first period of soils of *Poncirus trifoliata*. The same change was positive at 1 % significance level in *Troyer citrange*. According to these results, it can be suggested that *Poncirus trifoliata* rootstocks, which can uptake more Na^+ ions from soils compared to *Troyer citrange* rootstock, can prevent the increase of pH due to Na^+. Also, the reason for the positive relationship between pH and the levels of applied salt in *Troyer citrange* can be due to this rootstocks significant uptake of Na^+ from the soil.

Na^+ concentrations in soils belonging to *Poncirus trifoliata* rootstock was less in both periods compared to *Troyer citrange* rootstock. Okur et al. [11] and Anac et al. [6] suggested that this difference resulted from the nutrition properties of rootstocks. *Poncirus trifoliata* rootstock can uptake more Na^+ ion from soil. Can et al. [12] determined that the value of leaf area index was 1.3 in Satsuma budded onto *Poncirus trifoliata* rootstock and 1.8 in Satsuma budded onto *Troyer citrange* rootstock. When the salination rose to 6.5 dS m^{-1}, these values were 0.7 and 1.0 for *Poncirus trifoliata* and *Troyer citrange*, respectively. In this case, *Troyer citrange* rootstock, having more leaf area, will uptake more water from the soil and cause the moving of saline waters towards the rooting zone.

In the soil samples taken in August, it was determined that the negative relationships at 1 % significance level among the distance from stem and total soluble salt content, EC_e values, the concentrations of Na^+ and Cl^-.

Conclusion

During the first period, the total soluble salts, EC_e, Na_e^+ and Cl_e^- values for both rootstocks decreased as the distance of the stem gradually increased. In addition, these parameters also decreased depending on the increased depth. Salt accumulations can occur at the soil surface (first distance and first depth), because of upward water flow induced by capillary action and evaporation from the soil surface in the first period. In the soils of the second period (October), soil salinity increased as the distance of drippers and the soil depth increased. The characteristic leaching area occurred just under the drippers caused by the moving of salts from this region and accumulating in the outside of the leaching area throughout laterals. In addition to this, it can be said that an average of 63 mm of rain fell in the second sampling period from October and leached the salts to deeper zones and carried throughout the laterals. Accumulation of salinity at the bottom of the surface (second depth) occurs as the salts are left behind by water uptake and never washed out.

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