Chapter 35
Salt Response of Some Halophytes with Potential Interest in Reclamation of Saline Soils: Gas Exchange, Water Use Efficiency and Defence Mechanism

Hans-Werner Koyro, Salma Daoud, and M. Cherif Harrouni

Abstract Most models about global changes predict the development of salt deserts with strongly degraded vegetation, unhealthy living conditions and negative impact on economic goods. The reservoir of freshwater on earth (especially in arid zones) is not sufficient to ensure the feeding of manhood in future. Furthermore, the substitution of freshwater against saline sources in combination with unprofessional artificial irrigation systems leads to an increasing destruction of useful areas and to strong economic damages. The consecutive increase of soil salinity is a threat for the productive land because most crops have only a low degree of salt resistance. In future, halophytes, plants with a natural high salt resistance, can play a major role for the rehabilitation and economic use of salt-affected habitats. Halophytic ecosystems present a high productivity and can be the base for a sustainable agriculture on saline soils.

A precondition is the extension of the up to now incoherent knowledge about the ecology of halophytes, their economic potential and – for the warranty of a sustainable use – also about their individual mechanisms of resistance. The physiological studies with the sea water irrigation system shown in this chapter have the potential to provide highly valuable means of detecting individual mechanisms of species against NaCl toxicity and may also provide opportunities for the comparison and screening of different varieties for their adaptation to salinity

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(QCS for cash crop halophytes). After the selection of halophytic species suited for a particular climate and for a particular utilisation, greenhouse experiments at the local substrates (and climatic conditions) to select and propagate promising sites have to be started.

Worldwide, initiatives are being undertaken to develop saline vegetable crops, as well as crops for fuel and fibre, but the use of halophytes is still in the early stages of development.

Keywords Gas exchange • Halophytes • Salt injury • Salt tolerance • Water relations

35.1 Introduction

Seven percent of the land's surface and 5% of cultivated lands are affected by salinity (Ghassemi et al. 1995; Szabolcs 1994), with salt stress being one of the most serious environmental factors limiting the productivity of crop plants. When soils in arid regions of the world are irrigated, solutes from the irrigation water can accumulate and eventually reach levels that have an adverse affect on plant growth. Of the current 230 million ha of irrigated land, 45 million ha are salt-affected (19.6%) and of the 1,500 million ha under dryland agriculture, 32 million ha are salt-affected to varying degrees (2.1%). There are often no sufficient reservoirs of freshwater available, and most of the agronomically used irrigation systems are leading to a permanent increase in the soil salinity and step by step to growth conditions inacceptable for most of the conventional crops. Significant areas are becoming unusable each year. It is a worldwide problem, but most acute in Australasia (3.1 million ha), the Near East (1,802 million ha) and Africa (1,899 million ha), North and Latin America (3.963 million ha) and to an increasing degree also in Europe (2.011 million ha) of salt-affected soils (FAO 2008). Despite advances in increasing plant productivity and resistance to a number of pests and diseases, improving salt tolerance in crop plants remains elusive, mainly because salinity simultaneously affects several aspects of plant physiology.

In contrast to crop plants, there exist specialised plants that thrive in the saline environments along the seashore, in estuaries and saline deserts. These plants, called halophytes, have distinct physiological and anatomical adaptations to counter the dual hazards of water deficit and ion toxicity. Salinity can affect any process in the plant's life cycle, so that tolerance will involve a complex interplay of characters. New insights into the mechanisms by which plants achieve this have emerged from research projects investigating details of the physiology and biochemistry of salt tolerance. Unfortunately, there are few investigations which combine studies of growth and other measurements on both biophysical and biochemical plant characteristics. Such joint investigations will be particularly important in the discovery of traits which present the ability to maintain high plant productivity in saline environments.

The sustainable use of halophytic plants is a promising approach to valorise strongly salinised zones unsuitable for conventional agriculture and mediocre waters (Boer and Gliddon 1998; Lieth et al. 1999). There are already many halophytic species used for economic interests (human food, fodder) or ecological reasons (soil desalinisation, dune fixation, CO_2-sequestration). However, the wide span of halophyte utilisation is not yet explored even to a small degree.

35.1.1 Halophytes: Plants Able to Complete Their Life Cycle on Saline Substrates

Saline conditions reduce the ability of plants to absorb water, causing rapid reductions in growth rate, and induce many metabolic changes similar to those caused by water stress (Epstein 1980).

Halophytes are plants, able to complete their life cycle in a substrate rich in NaCl (Schimper 1891). One of the most important properties of halophytes is their salinity tolerance (Lieth 1999). This substrate offers for obligate halophytes advantages for the competition with salt-sensitive plants (glycophytes). There is a wide range of tolerance among the 2,600 known halophytes (Pasternak 1990; Lieth and Menzel 1999). However, information about these halophytes needs partially careful checking. A precondition for a sustainable utilisation of suitable halophytes is the precise knowledge about their salinity tolerance and the various mechanisms enabling a plant to grow at (their natural) saline habitats (Marcum 1999; Warne et al. 1999; Weber and D'Antonio 1999; Winter et al. 1999). This chapter concentrates on the eco-physiological mechanisms of salt tolerance.

35.1.2 Complexity of Salt Tolerance

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 (Läuchli and Epstein 1990; Maas 1990). Numerous attempts have been made to improve the salt tolerance of crops by traditional breeding programmes. However, commercial success has been very limited due to the complexity of the trait: salt resistance is genetically and physiologically complex (Flowers 2004). At present, major efforts are being directed towards the genetic transformation of plants in order to raise their tolerance (Borsani et al. 2003).

Improving salt resistance of crop plants is of major concern in agricultural research. A potent genetic source for the improvement of salt resistance in crop plants resides among wild populations of halophytes (Glenn et al. 1999; Serrano et al. 1999). 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.

35.1.3 *State of the Art in Sustainable Utilisation with Saline Irrigation Waters*

There are already several examples known for the utilisation of halophytes for industrial, ecological or agricultural purposes (Lieth et al. 1999). Because of their diversity, halophytes have been tested as vegetable, forage and oilseed crops in agronomic field trials (Koyro et al. 2006; Hoek 2008). The most productive species yield 10–20 t ha^{-1} of biomass on sea water irrigation, equivalent to conventional crops. The oilseed halophyte, *Salicornia bigelovii*, yields 2 t ha^{-1} of seeds containing 28% oil and 31% protein, similar to soybean yield (Glenn et al. 1999).

In several countries, specific plant species are used for waste water treatment. Some halophytes can be used for bioremediation of salt-contaminated soils, and even pharmaceutical values of their plant products are described (Lieth et al. 1999; Hoek 2008; Rozema and Flowers 2008). Halophyte forage and seed products already replaced conventional ingredients in animal feeding systems, with some restrictions on their use due to high salt content and anti-nutritional compounds present in some species.

35.2 The Quick Check System

It is – without doubt – necessary to develop sustainable biological production systems which can tolerate higher water salinity because freshwater resources will become limited in near future (Lieth 1999). A precondition is the identification and/or development of salinity tolerant crops. An interesting system approach lines out that after halophytes are studied in their natural habitat and a determination of all environmental demands has been completed, the selection of potentially useful plants should be started (Lieth 1999). The first step of this identification list contains the characterisation and classification of the soil and climate, under high potentially useful halophytes grow. Only artificial conditions in sea water irrigation systems in a growth cabinet under photoperiodic conditions offer the possibility to study potentially useful halophytes under reproducible experimental growth and substrate conditions. The supply of different degrees of sea water salinity (0, 25, 50, 75, and 100% (and if necessary higher) sea water salinity) to the roots in separate systems under otherwise identical or/and close to natural conditions gives the necessary preconditions for a comparative study in a quick check system (QCS) for potential cash crop halophytes (US Salinity Laboratory Staff 1954). The experiments of the QCS started off at steady state conditions in a gravel/hydroponic system imitating the climatic conditions of subtropical dry regions (Fig. 35.1, Koyro and Huchzermeyer 1999a). It is well known that salinity tolerance depends on the stage of development and period of time over which the plants have grown in saline conditions (Munns 2002). Plants were exposed to salinity in the juvenile state of development and were studied until achieving the steady state of adult plants. Variable applicable QCS seems to be valuable for the selection of useful plants, and it suggests itself as a first

Fig. 35.1 Quick check systems of several halophytes under photoperiodic conditions in a growth cabinet; (**a**) *Chenopodium quinoa*, (**b**) *Beta vulgaris ssp. Maritime*, (**c**) *Spartina townsendii*, (**d**) *Inula critmoides*, (**a**, **b**, and **d**) Gravel/hydroponic quick check system with automatic drip irrigation. (**c**) Intertidal irrigation (alternating water level) quick check system

step for the controlled establishment of cash crop halophytes because it provides detailed information about three major goals as they are the threshold of salinity tolerance at idealised growth conditions, how to uncover the individual mechanisms for salt tolerance and about the potential of utilisation for the pre-selected halophytic species (cash crop halophytes).

35.3 Threshold of Salinity Tolerance

In correspondence with the definition for the threshold of salinity tolerance according to Kinzel (1982), the growth reaction and the gas exchange are used during the screening of halophytes as objective parameters for the description of the actual condition of a plant (Ashraf and O'Leary 1996). There are now reliable information available about studies with several halophytic species from different families such as *Chenopodium quinoa* (Figs. 35.1a and 35.2a), *Aster tripolium*, *Plantago* cf. *coronopus*, *Beta vulgaris* ssp. *maritima* (Figs. 35.1b and 35.2b), *Batis maritima*, *Puccinellia maritima*, *Spartina townsendii* (Figs. 35.1c and 35.2c), *Atriplex nummularia*,

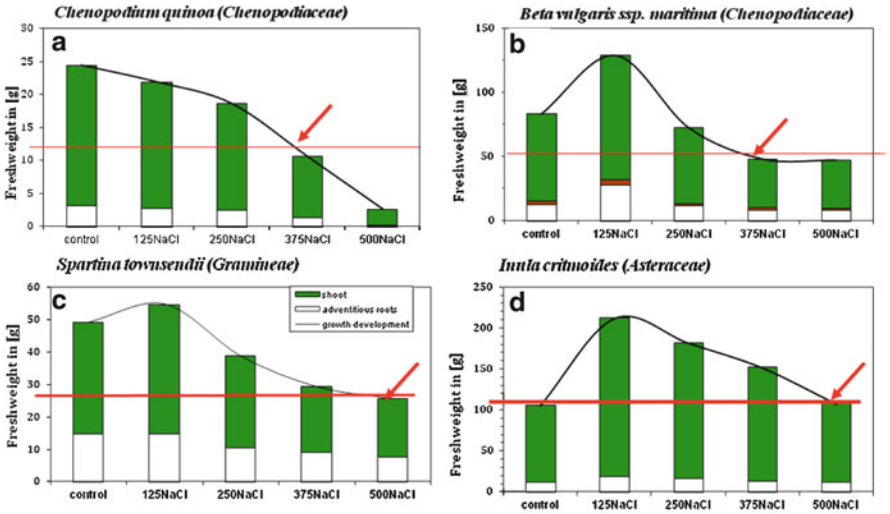


Fig. 35.2 Development of the plant freshweight at treatments with different percentages of sea water salinity. The crossover of the red and the black lines reflects the NaCl salinity where the growth depression falls down to 50% of the control plant (threshold of NaCl salinity according to Kinzel, 1982). (**a**) *Chenopodium quinoa* (*Chenopodiaceae*) 75% sea water salinity; (**b**) *Beta vulgaris* ssp. *maritima* (*Chenopodiaceae*): 75% sea water salinity; (**c**) *Spartina townsendii* (*Gramineae*): 100% sea water salinity; (**d**) *Inula critmoides* (*Asteraceae*): 100% sea water salinity. 0% sea water salinity = control, 25% = 125NaCl, 50% = 250NaCl, 75% = 375NaCl and 100% = 500NaCl

Atriplex leucoclada, *Atriplex halimus*, *Laguncularia racemosa*, *Limoneastrum articulatum*, *Sesuvium portulacastrum* and *Inula critmoides* (Figs. 35.1d and 35.2d) (Pasternak 1990; Koyro and Huchzermeyer 1997, 1999a, 2004b; Koyro et al. 1999; Lieth and Menzel 1999; Koyro 2000). The substrate concentration leading to a growth depression of 50% (refer to freshweight, in comparison to plants without salinity) is easy to calculate with the QCS (by extrapolation of the data), and it leads to a precise specification of a comparative value for the threshold of salinity tolerance (Fig. 35.2a–d). Dramatic differences are found between halophytic plant species. The threshold of salinity tolerance amounts to 350 mol*m^{-3} NaCl in *Chenopodium quinoa* and *Beta vulgaris* ssp. *maritima* and 500 mol*m^{-3} in *Spartina townsendii* and in *Inula critmoides* (Fig. 35.2). These results prove that it is essential to quantify differences in salinity tolerance between halophytic species as one basis for assessment of their potential of utilisation.

35.4 Morphological Structures to Reduce Salt Concentrations

In many cases various mechanisms and special morphological structures are advantageous for halophytes since they help to reduce the salt concentrations especially in photosynthetic or storage tissue and seeds. Salt glands may eliminate large quantities of

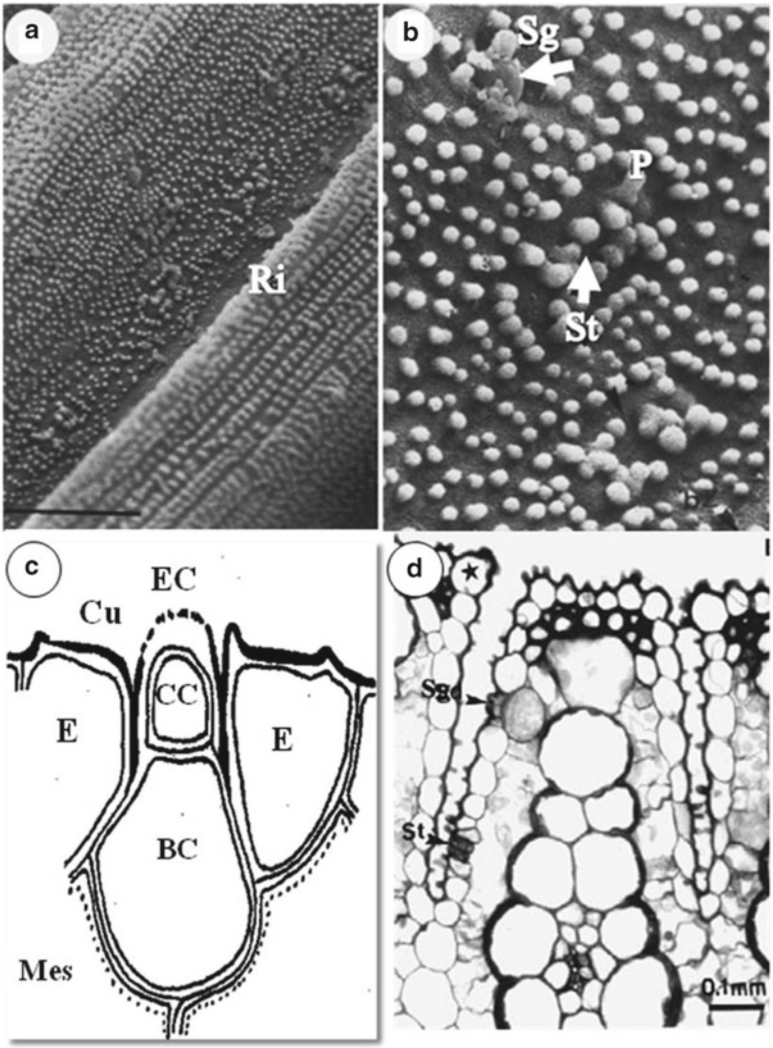


Fig. 35.3 Salt glands on a leaf of *Spartina townsendii*. The adaxial surface of the blade is increased enormously by ridges running from just above the pulvinus to the apex (**a**). These provide an increased assimilatory surface in addition to protection of the stomata (**a**, **b** and **d**). Latter one is also reached by papillae and waxy coating on the surface (**b**). Salt glands (**c** and **d**) take the place of the water pores found in many submerged plants. Scheme of a salt gland of *Spartina townsendii* (**c**). *Sg* salt gland, *St* stomata, *CC* capcell, *Ec* extracellular channel, *BC* basal cell, *P* papillae and waxy coating, *E* Epidermis, *M* mesophyll

salt by secretion to the leaf surface. This secretion appears in complex multicellular organs, for example, in *Avicennia marina* or by simple two cellular salt glands, for example, in *Spartina townsendii* (Fig. 35.3, Sutherland and Eastwood 1916; Walsh 1974; Koyro and Stelzer 1988; Koyro et al. 1997; Marcum et al. 1998). Several halophytes can reduce the salt concentrations in vital organs by accumulation in bladder hairs (*Atriplex halimus*, *Leptochloa fusca* (L.), *Halimione portulacoides*), enhancing the LMA (leaf mass to area ratio, e.g. by *Suaeda fruticosa*, *Salicornia europaea*, *Salsola kali*, *Sesuvium portulacastrum*), establishing apoplastic barriers (Freitas and Breckle 1992, 1993a, b; Hose et al. 2001), translocating NaCl into special organs (z.B. *Kandelia candel* L.), using of ultrafiltration at the root level to exclude salt (*Avicennia marina*, *Sonneratia alba*) or shedding of old leaves (*Beta vulgaris* ssp. *maritima*, see literature in Marschner 1995; Schroeder 1998; Glaubrecht 1999; Koyro 2002).

35.5 Screening Procedure

However, many halophytic species can tolerate high sea water salinity without possessing special morphological structures. To achieve salt tolerance, three interconnected aspects of plant activity are important for plants with or without salt glands. Damage must be prevented, homeostatic conditions must be re-established and growth must resume. Growth and survival of vascular plants at high salinity depends on adaptation to both low water potentials and high sodium concentrations, with high salinity in the external solution of plant cells producing a variety of negative consequences. It is the exception that a single parameter is of major importance for the ability to survive at high NaCl salinity. A comprehensive study with the analysis of at least a combination of several parameters is a necessity to get a survey about mechanisms constitution leading at the end to the salinity tolerance of individual species. These mechanisms are connected to the four major constraints of plant growth on saline substrates:

(a) Water deficit
(b) Restriction of CO_2 uptake
(c) Ion toxicity
(d) Nutrient imbalance

Plants growing in saline habitats face the problem of having low water potential in the soil solution and high concentrations of potentially toxic ions such as chloride and sodium. Salt exclusion minimises ion toxicity but accelerates water deficit and diminishes indirectly the CO_2 uptake. Salt absorption facilitates osmotic adjustment but can lead to toxicity and nutritional imbalance.

The presence of soluble salts can affect growth in several ways (Mengel and Kirkby 2001). In the first place plants may suffer from water stress, secondly high concentrations of specific ions can be toxic and induce physiological disorders and thirdly intracellular imbalances can be caused by high salt concentration.

Terrestrial plants at saline habitats are often surrounded by low water potentials in the soil solution and atmosphere. Plant water loss has to be minimised under these circumstances, since biomass production depends mainly on the ability to keep a high net photosynthesis by low water loss rates. Therefore, one crucial aspect of the screening procedure is the study of growth reduction and leaf (plant) water

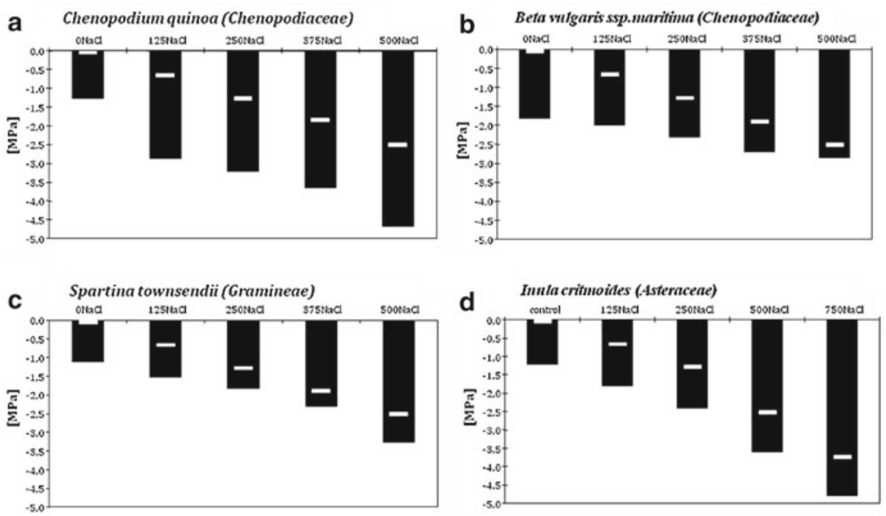


Fig. 35.4 Leaf water potentials (MPa) of (**a**) *Chenopodium quinoa* (*Chenopodiaceae*), (**b**) *Beta vulgaris* ssp. *maritima* (*Chenopodiaceae*) (**c**) *Spartina townsendii* (*Gramineae*) and (**d**) *Inula critmoides* (*Asteraceae*). The white lines in the bars mark the water potentials in the nutrient solutions. Leaf water potentials were always lower than the assigned nutrient solution potential. The difference between water potentials in the leaves and in the nutrient solutions decreased with increasing NaCl salinity. 0% sea water salinity = control, 100% sea water salinity = sea water salinity

potential especially at the threshold of salinity tolerance (Fig. 35.4). Water deficit is one major constraint at high salinity and can lead to a restriction of CO_2 uptake. The balance between water loss and CO_2 uptake is another basis for assessment of their potential of utilisation. Additionally, it helps to find weak spot in the mechanisms of adjustment (of photosynthesis) to high salinity.

35.6 Balance of Water Loss and CO_2 Uptake

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 (Maathuis and Amtmann 1999). Terrestrial plants at saline habitats are often surrounded by low water potentials in the soil solution and atmosphere. It is important to prevent water loss by transpiration from being higher than the influx rate. This is only possible if the water potential is lower in the plant than in the soil.

Data of leaf water potentials demonstrate clearly that leaf water potential of halophytes does not correlate alone as a single factor with salinity tolerance (Koyro 2006). They do not correlate at all with the respectively existing salt resistance in species such as *Chenopodium quinoa*, *Beta vulgaris* ssp. *maritima*, *Spartina townsendii* and

Sesuvium portulacastrum. All these species have a sufficiently low water potential even at high salinity although their salt resistance (definition see above) varies between 0.5 times (*Chenopodium quinoa*) and 1.5 times sea water salinity (*Sesuvium portulacastrum*). Furthermore, the osmotic potentials of all four halophytes (and many other) were sufficiently low to explain the full turgescence of the leaves at all salinity levels.

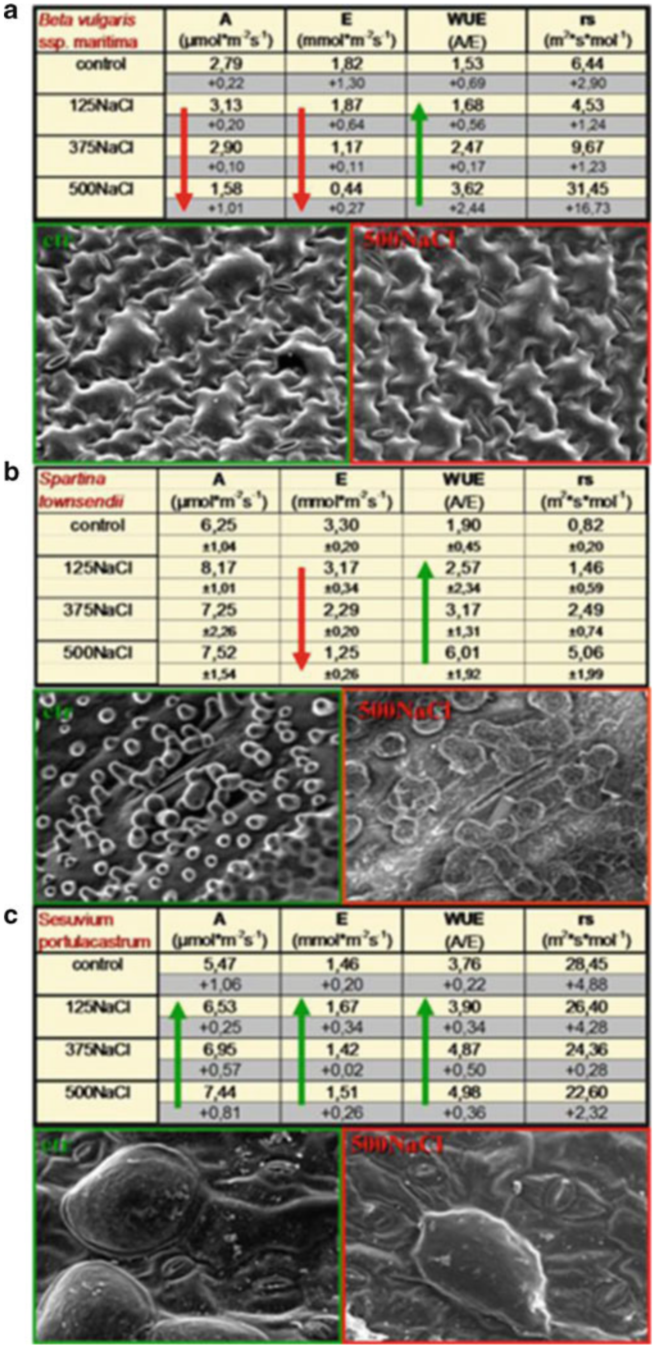
However, plant water loss has to be minimised at low soil water potentials, since biomass production depends mainly on the ability to keep a high net photosynthesis by low water loss rates. In this field of tension, biomass production of a plant has always to be seen in connection to the CO_2/H_2O-gas exchange which can be estimated based on water use efficiency (WUE) of photosynthesis. A critical point for the plant is reached if the CO_2-fixation (apparent photosynthesis) falls below the CO_2-production (compensation point). Therefore, one crucial aspect of the screening procedure is the study of growth reduction, water consumption and net photosynthesis especially at the threshold of salinity tolerance (Fig. 35.5).

Many plants such as *Chenopodium quinoa*, *Aster tripolium*, *Beta vulgaris* ssp. *maritima* or *Spartina townsendii* reveal a combination of low (but positive) net photosynthesis, minimum transpiration, high stomatal resistance and minimum internal CO_2-concentration at their threshold salinity tolerance (Koyro and Huchzermeyer 2004a). However, there is a big bandwidth between halophytes. Especially, succulent halophytes such as *Sesuvium portulacastrum* and *Avicennia marina* have alternatives if the water balance is still positive (water uptake minus water loss) and not the limiting factor for photosynthesis. In case of *Sesuvium* net photosynthesis and WUE increase but stomatal resistance decrease. These results show that it is quite important to describe the regulation of gas exchange at high salinity in strong reliance with other parameters (such as water relations).

35.7 Ion Excess, Deficiency and Imbalance

In principle, salinity tolerance can be achieved by salt exclusion or salt inclusion. Several physiological mechanisms are described in literature which avoid salt injury (and to protect the symplast) are known as major plant responses to high NaCl salinity (Marschner 1995; Flowers et al. 1997; Mengel and Kirkby 2001; Munns 2002).

Useful parameters for screening halophytes should base on the major plant responses to high NaCl salinity (Volkmar et al. 1998). It seems to be essential that such a screening system should include salt-induced morphological changes such as succulence and LAR (leaf mass to area ratio, Koyro 2002), growth, water relations, gas exchange and composition of minerals (and compatible solutes) at different parts of the root system and in younger and older leaf tissues. The measurement of such general scientific data at plant, organ or tissue level reveals general trends – but since these represent a mean behaviour of several cell types, much information on single-cell adjustment are lost. They cannot give sufficient information about the compartmentation inside a cell or along a diffusion zone in a root apoplast or about



Beta vulgaris ssp. *maritima*	A (µmol*m^{2}s^{1})	E (mmol*m^{2}s^{1})	WUE (A/E)	rs (m^{2}*s*mol^{1})
control	2,79	1,82	1,53	6,44
	+0,22	+1,30	+0,69	+2,90
125NaCl	3,13	1,87	1,68	4,53
	+0,20	+0,64	+0,56	+1,24
375NaCl	2,90	1,17	2,47	9,67
	+0,10	+0,11	+0,17	+1,23
500NaCl	1,58	0,44	3,62	31,45
	+1,01	+0,27	+2,44	+16,73

Spartina townsendii	A (µmol*m^{2}s^{1})	E (mmol*m^{2}s^{1})	WUE (A/E)	rs (m^{2}*s*mol^{1})
control	6,25	3,30	1,90	0,82
	±1,04	±0,20	±0,45	±0,20
125NaCl	8,17	3,17	2,57	1,46
	±1,01	±0,34	±2,34	±0,59
375NaCl	7,25	2,29	3,17	2,49
	±2,26	±0,20	±1,31	±0,74
500NaCl	7,52	1,25	6,01	5,06
	±1,54	±0,26	±1,92	±1,99

Sesuvium portulacastrum	A (µmol*m^{2}s^{1})	E (mmol*m^{2}s^{1})	WUE (A/E)	rs (m^{2}*s*mol^{1})
control	5,47	1,46	3,76	28,45
	+1,06	+0,20	+0,22	+4,88
125NaCl	6,53	1,67	3,90	26,40
	+0,25	+0,34	+0,34	+4,28
375NaCl	6,95	1,42	4,87	24,36
	+0,57	+0,02	+0,50	+0,28
500NaCl	7,44	1,51	4,98	22,60
	+0,81	+0,26	+0,36	+2,32

Fig. 35.5 Influence of NaCl salinity on the apparent photosynthesis (*A*), the adaxial transpiration (*E*), the water use efficiency (WUE) and the stomatal conductance (rs) of *Beta vulgaris* ssp. *maritima* (**a**), *Spartina townsendii* (**b**) and *Sesuvium portulacastrum*. The ultrastructures (SEM-micrograph) of the stomata on the leaf surfaces (*left side* controls, *right side* sea water salinity treatments) are presented next to the responding table

ultrastructural changes such as apoplastic barriers (Hose et al. 2001). The collection of scientific data should be completed if necessary (to uncover the individual mechanisms for salt tolerance) by a special physiological research at single-cell level supplemented optionally by methods such as the analysis of the gene expression and its genetic basis (genomics and proteomics, Winicov and Bastola 1997, 1999; Winicov 1998).

The general scientific data give an impression of various mechanisms of adaptation to high NaCl salinity. Besides water stress and ion-specific toxic physiological disorders on tissue level, intracellular ionic imbalances (K^+, Ca^{2+} and Mg^{2+}) can be caused by high salt concentrations (Mengel and Kirkby 2001). The capacity of plants to maintain K^+-homeostasis and low Na^+ concentrations in the cytoplasm appears to be one important determinant of plant salt tolerance (Yeo 1998; Läuchli 1999). A possibility to find such limiting factors is the study of the relations inside single cells such as the compartmentation between cytoplasm and vacuole, the distribution of elements in different cell types or along a diffusion zone in a root apoplast and ultrastructural changes.

Beta vulgaris ssp. *maritima* and *Spartina townsendii* keep Na and Cl concentrations low (Fig. 35.6) in their young growing tissues (such as juvenile leaves) and in their storage organs (such as taproot or rhizome). However, *Beta vulgaris* ssp. *maritima* is a typical Cl-includer and *Spartina townsendii* a typical Cl-excluder with high Na-accumulation in the leaves. Both species seem to react similar to salinity with changes of leaf water potential, gas exchange and nutrients (Koyro and Huchzermeyer 2004a). However, this number of partially congruent or complementary results does not allow concluding analogical intracellular relations. The comparison of their intracellular ionic balance will be used to demonstrate the necessity of special physiological investigations.

In contrast to water stress effects, occurring in the meristematic region of younger leaves, salt toxicity predominantly occurs in adventitious roots and mature leaves (Mengel and Kirkby 2001). Furthermore, most of the Na and Cl are stored mainly in the shoot of halophytes such as *Beta vulgaris* ssp. *maritima* and *Spartina townsendii* leading to a growth reduction of the above ground parts much higher than of the root (Koyro 2000; Koyro and Huchzermeyer 2004b). These changes can be interpreted as signs of a critical load. Therefore, to distinguish between the individual mechanisms of salinity tolerance, further investigations of the intracellular ionic balance were performed first of all at epidermal leaf cells (the end of the transpiration stream) of these both species.

The single cell data of the vacuolar and cytoplasmatic composition in cells of the upper leaf epidermis are summarised for the controls and the high-salinity treatments (at sea water salinity) in Table 35.1. The intracellular composition of the leaf epidermal cytoplasm and vacuoles of controls of *Beta vulgaris* ssp. *maritima* and *Spartina townsendii* show some more congruities of both species. The epidermal vacuoles of controls of both species contain most of the elements (with the exception of P) in higher concentrations as the cytoplasm indicating the overall picture of a vacuolar buffer. The leaf vacuoles in its entirety can be described as a voluminous potassium pool with high storage capacity for sodium and chloride. This pool is

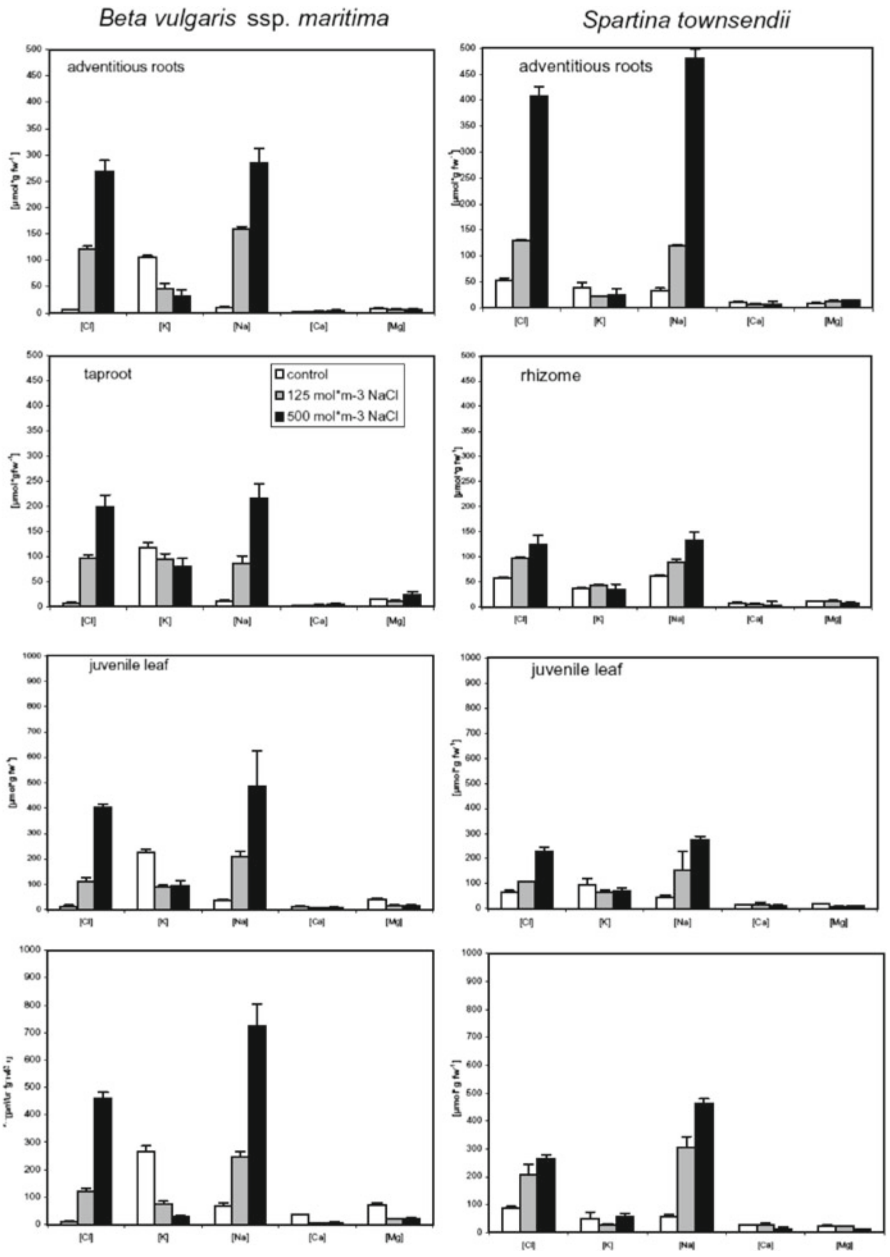


Fig. 35.6 Chlorine, potassium, sodium, calcium and magnesium concentrations in mol m^{-3} in different tissues of *Beta vulgaris* ssp. *maritima* and *Spartina townsendii*

Table 35.1 Chlorine, phosphorus, sulphur, sodium, magnesium, potassium and calcium concentrations in mol * m^{-3} (measured with EDX-analysis in bulk frozen tissues) in the vacuoles and in the cytoplasm of adaxial epidermis cells of *Beta vulgaris* ssp. *maritima* and *Spartina townsendii*

Adaxial leaf epidermis	Vacuole				Cytoplasm			
	Control		480 NaCl		Control		480 NaCl	
Beta vulgaris ssp. maritima								
Cl	22.6	±4.7	654.3	±54.8	0.0		<5	
P	22.2	±4.6	6.4	±4.2	58.2	±8.4	28.1	±6.7
S	40.4	±7.9	2.7	±1.9	10.5	±4.0	<5	
Na	12.2	±3.1	724.9	±65.1	<5		<5	
Mg	24.0	±1.2	4.3	±1.7	<5		<5	
K	282.5	±14.7	9.3	±6.1	88.9	±9.5	66.7	±7.2
Ca	<5		<5		<5		<5	
Spartina townsendii								
Cl	21.2	±3.2	324.3	±64.8	<5		<5	
P	11.1	±1.1	5.3	±2.1	81.5	±6.8	71.6	±10.8
S	24.4	±6.9	20.8	±2.6	8.8	±3.0	<5	
Na	16.4	±3.1	521.0	±54.9	<5		15.3	±3.2
Mg	29.6	±2.1	18.8	±1.6	<5		<5	
K	212.5	±34.8	71.5	±6.9	92.7	±12.7	78.4	±6.9
Ca	<5		<5	±2.1	<5		<5	

needed in case of high NaCl salinity for the maintenance of the K-homeostasis in the cytoplasm. The dominant elements in the cytoplasm were P and K. The K-concentrations were in the epidermal cytoplasm of control plants in an ideal range for enzymatic reactions (Wyn Jones et al. 1979; Wyn Jones and Pollard 1983; Koyro and Stelzer 1988).

It is obvious that sea water salinity leads to a decrease of P, S, Mg and K in the epidermal vacuoles of both species. The remaining K, S and Mg concentrations were only in *Spartina* two-digit and especially for K much higher as in *Beta*. The vacuolar buffer of the latter one seems to be exhausted.

NaCl salinity led to a significant decrease of the K and P concentrations especially in the cytoplasm of *Beta* and to a breakdown of the homeostasis (Koyro and Huchzermeyer 1997). This result points at a deficiency for both elements in the cytoplasm. Additionally, the concentrations of sodium and chlorine were at high NaCl salinity below 5 mol m^{-3} in the cytoplasm of the epidermal cytoplasm, and the gradients between cytoplasm and vacuole were higher in comparison with the results of *Spartina*. In summary these results support the hypothesis that the sea beet does not sustain ion toxicity but ion deficiency! It is hypothesised that such low K^{+} levels in the cytoplasm can lead to a reduction of protein synthesis which is of utmost importance in the process of leaf expansion (Mengel and Kirkby 2001). One possible consequence is the supply of sufficient fertilisers (especially K and P) at high NaCl salinity to reduce the symptoms of K- and P-deficiency in Beta.

The salt-induced reductions of the cytoplasmic K and P concentrations were much less pronounced in Spartina as in Beta. The results of Spartina point at a working system to keep ionic homeostasis. However, there was one important exception: The sodium concentration increased significantly in the epidermal cytoplasm. Sodium could (try to) substitute potassium in its cytoplasmic functions or it could be the first sign of intoxication.

The results and interpretations are in agreement with the hypothesis that plant growth is affected by ion imbalance and toxicity and probably leads to the long-term growth differences between the salt-tolerant and salt-sensitive species.

However, Beta and Spartina are also two excellent examples how important it can be to validate intracellular ionic imbalances (K^+, Ca^{2+} and Mg^{2+}) at high salt concentrations to uncover the individual mechanisms for salt tolerance and to understand the threshold levels of individual species.

35.8 Compatible Solutes

A metabolic response to salt stress especially in salt excluding species is the synthesis of compatible osmolytes. These mediate osmotic adjustment and therefore achieve a low water potential and/or a charge balance, protect subcellular structures and reduce oxidative damage caused by free radicals, produced in response to high salinity (Hong et al. 1992; Hare et al. 1998). The solute potential in these species is decreased by the synthesis of organic solutes such as sugar alcohols (e.g. mannitol in leaves of L*aguncularia racemosa*), soluble carbohydrates (e.g. sucrose in the taproots of *Beta vulgaris* ssp. *maritima*) and organic acids (including amino acids) or by reducing the matrical potential (e.g. with soluble proteins in leaves of *Beta vulgaris* ssp. *maritima*; results not shown). However, the synthesis of organic solutes is energy demanding (s.a. also includers), and the formation of these solutes decreases the energy status of the plant (Yeo 1983). Thus, for plant survival, growth depression in excluder species can be a necessary compromise in Na^+ and /or Cl^- excluding species and not a sign of toxicity or nutrient imbalance (Koyro and Huchzermeyer 1999b).

35.9 Protection of Metabolism

Mechanisms for tolerance of the salt-specific effects of salinity are of two main types: those minimising the entry of salt into the plant and those minimising the concentration of salt in the cytoplasm (Fig. 35.4). Root and leaf cytosolic Na^+ and Cl^- concentrations are in the order of 10–30 mM (Tester and Davenport 2003; Wyn Jones and Gorham 2002; Koyro and Huchzermeyer 2004b).

The destruction of metabolism by Na^+ or Cl^- has to be avoided if plants have to grow on saline habitats. Therefore, the protection of the responsible enzymes is of major importance. The ability of plant cells to maintain low cytosolic sodium concentrations is an essential process for halophytes (Borsani et al. 2003). Leaves being fed by the transpiration stream receive large quantities of sodium, which must be regulated. Plant cells respond to salt stress by increasing sodium efflux at the plasma membrane and sodium accumulation in the vacuole. Thus, the proteins, and ultimately genes, involved in these processes can be considered as salt tolerance determinants. The cloning experiments of Na^+/H^+ antiporter have demonstrated the role of intracellular sodium (Ohta 2002) compartmentalisation in plant salt resistance. Such compartmentalisation of sodium and chloride in leaf vacuoles can only be attained by an active transport into the vacuole and low tonoplast permeability to these ions.

The transport of ions across the plasma membrane and tonoplast requires energy, which is provided by vacuolar and plasma membrane ATPase (Gordon-Weeks et al. 1997; Koyro and Huchzermeyer 1997; Leigh 1997; Leigh and Sanders 1997). Sodium ions exchanged for hydrogen ions across membrane Na^+/H^+ antiporter take advantage of a proton gradient formed by these pumps. Salt stress was shown to increase Na^+/H^+ activity in glycophytes and halophytes (Apse and Blumwald 2002). The activation of such antiporter is likely to be operating to reduce sodium toxicity in salt-tolerant plants under saline conditions.

35.10 Overview of Results Presented

The results presented in this chapter contain a lot of information about the essential eco-physiological needs of several halophytes at high salinity. The very variable screening of individual species enables to study the characteristic combination of mechanisms against salt injury and the threshold of salinity tolerance. The so-called QCS can be modified to the special characteristics and needs of other species and is therefore useful to study a wide range of suitable halophytes. This screening procedure is a practical first step on the selection of economically important cash crop halophytes.

For future studies on utilisation potentials of halophytes, precise data about the ecological demands of halophytic species are required. Comparative physiological studies about salinity tolerance are essential. A precondition for this demand is a precise specification of a comparative value for halophytic species as shown in this chapter. The literature has to be screened prior to the selection of priority species (potentially useful species) in order to get first-order information about their natural occurrence in dry or saline habitats, existing utilisation (because of their structure, chemical content or other useful properties), natural climatic and substrate conditions, water requirement and salinity tolerance. Soon after the selection of a priority species, the threshold of salinity should be determined according to Kinzel (1982) and Munns (2002). The characteristic major plant response has to be evaluated for precise information of eco-physiological demands. The data can build up a well-founded basis for the improvement of the utilisation potential. Additionally, research about the genetic composition of chromosomes mastering saline environment is also needed and bases on quantitative precise determination (Winicov and Bastola 1997, 1999; Winicov 1998).

35.11 Development of Cash Crop Halophytes

The physiological studies with the sea water irrigation system have the potential to provide highly valuable means of detecting individual mechanisms of species against NaCl stress and may also provide opportunities for the comparison and screening of different varieties for their adaptation to salinity (QCS for cash crop halophytes). After the selection of halophytic species suited for a particular climate and for a particular utilisation, greenhouse experiments at the local substrates (and climatic conditions) to select and propagate promising sites (Isla et al. 1997) have to be started. This must be followed by studies with Lysimeters on field site to study the water consumption and ion movements. Last but not the least: A design for a sustainable production system in plantations at coastal areas or at inland sites (e.g. for economical use) needs to be developed.

35.12 Future Perspective

Time is running fast and the last decade has witnessed, especially in the arid and semi-arid regions, a sharp increase in losses of arable land due to salinisation. As shown above, salinity is an ever-present threat to crop yield, especially in countries where irrigation is an essential aid to agriculture (Flowers 2004). Irrigation farming is expanding fast, and many fields have reached a soil salinity level which prevents farmers from raising common crops. If we are not reacting soon, there is no further necessity to study salt-resistant genes because soil salinity worldwide will reach levels where no halophyte can grow at all.

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