CHAPTER 22

MECHANISMS OF CASH CROP HALOPHYTES TO MAINTAIN YIELDS AND RECLAIM SALINE SOILS IN ARID AREAS

HANS-WERNER KOYRO[1,3], NICOLE GEISSLER[1], SAYED HUSSIN[1] AND BERNHARD HUCHZERMEYER[2]

[1]*Institute for Plant Ecology, Justus-Liebig-University of Giessen, D-35392 Giessen,*
[2]*Botany Institute, Plant Developmental Physiology and Bioenergetics, Hannover University, D-30419 Hannover*
[3]*Corresponding author: Hans-Werner.Koyro@bot2.bio.uni-giessen.de*

1. INTRODUCTION

About 7% of the world's total land area is affected by salt, as is a similar percentage of its arable land (Ghassemi et al., 1995; Szabolcs, 1994) 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 (19.5 percent) and of the 1,500 million ha under dryland agriculture, 32 million are salt-affected to varying degrees (2.1 percent). There are often not 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 unacceptable 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 hectars), the Near East (1.802 million hectars) and Africa (1.899 million hectars), North and Latin America (3.963 million hectars) and to an increasing degree also in Europe (2.011 million hectares of salt-affected soils; FAO Land and Plant Nutrition Management Service). Although careful water management practices can avoid, or even reclaim damaged land, crop varieties (such as cash crop halophytes) that can maintain yields in

saline soils or allow the more effective use of poor quality irrigation water will have an increasing role in agricultural land use in near future.

In contrast to crop plants, there exist specialists 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. Research projects investigated details of the physiology and biochemistry of salt tolerance and also searched for methods to screen overall plant performance that could be used in breeding programs.

The sustainable use of halophytic plants is a promising approach to valorize strongly salinised zones unsuitable for conventional agriculture and mediocre waters (Boer & 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 jet explored even to a small degree.

2. DEFINITIONS OF THE TERMS HALOPHYTE AND SALINITY TOLERANCE

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 2600 known halophytes (Pasternak, 1990; Lieth & 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 & Dántonio, 1999; Winter et al., 1999). The many available definitions especially for salinity tolerance or threshold of salinity tolerance impede a uniform description and complicate the comparison between species:

 a) Phytosociologists are using this term only for plants growing natural in saline habitats. In order to get first information on salinity tolerance phytosociological vegetation analysis is very helpful and salinity tolerance numbers are widely applied for qualitative approximations (Ellenberg, 1974; Landolt, 1977).
 b) Another group of scientists describes salinity tolerance with polygonal diagrams of the mineral composition in the plants (see literature in Kinzel, 1982; Marschner, 1995).
 c) The threshold level of salinity tolerance is described in a further definition as the point (salt concentration) when the ability of plants to survive and to

reproduce is no longer given (Pasternak, 1990). This definition is a modification of the definition of halophytes presented above. Survival and reproduction of a plant are not always impeded at the same salinity level (Tazuke, 1997). However, the definition of Pasternak (1990) is still important for the interpretation of the ecological dissemination and can be used as a solid basis for physiological studies concerning the survival strategies of plants.

d) Generally, classification of the salinity tolerance (or sensitivity) of crop species is based on the threshold EC (electrical conductivity) and the percentage of yield decrease beyond threshold (Greenway & Munns, 1980; Marschner, 1995). Salinity tolerance is usually assessed as the percentage biomass production in saline versus control conditions over a prolonged period of time (Munns, 2002). The substrate-concentration leading to a growth depression of 50 % (refer to fresh weight, in comparison to plants without salinty) is widely used by ecophysiologists as a definition for the threshold of salinity-tolerance (Kinzel, 1982). This definition is based on the same background as the Michaelis/Menten factor, because it is as difficult to fix the upper limit of salinity tolerance, as it is to determine the lowest substrate concentration for an optimal enzymatic activity. The agreement to the above-mentioned growth depression is comparatively arbitrary, but it leads to a precise specification of a comparative value for halophytic species and is especially expressive for applied aspects such as economic potentials of suitable halophytes.

e) It is worth mentioning that there is another definition of salinity tolerance in use for glycophytic species. Especially in agriculture it is very common to speak of salinity tolerance if a variety of a glycophyte such as *Hordeum vulgare* survives at a slightly higher salinity level than another variety of the same species. However, the tolerated NaCl-substrate concentrations are in both variieties far beyond seawater salinity (Amzallag, 1994; Jeschke et al., 1995).

This paper concentrates on eco-physiological mechanisms. We will carry on using the term (threshold of) salinity tolerance for unambiguous understanding as defined in (d).

3. QUICK CHECK SYSTEM FOR THE SELECTION OF USEFUL PLANTS AND THE PHYSIOLOGICAL CHARACTERIZATION OF SALINITY TOLERANCE

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 which potentially useful halophytes grow.

The measurement of the EC (electrical conductivity in $[\mu S * cm^{-1}]$ offers a simple method for characterizing the salt content (Osmotic potential MPa = EC * - 0,036, Koyro & Lieth, 1998). A saline soil has an EC greater than 4 mmho $* cm^{-1}$ (equivalent to 40 mol $* m^{-3}$ NaCl; U.S. Salinity Staff, 1954; Koyro & Lieth, 1998) and is widely used for this purpose. Spatial variability in salt-affected fields is normally very high. Since the habitats are often complex and the concentrations vary with water content, the EC of saturation extract only is an insufficient indicator for salinity tolerance. Plant growth in saline soils can be influenced although the EC indicates no changes, because the actual salt concentration at the root surface can differ as compared to the bulk soil. The EC characterises only the total salt content but not changes in its spatial composition. The importance of micro-heterogenity of salinity and fertility for maintenance of the plant diversity was shown for example from several authors (Igartua, 1995; Abdelly et al., 1999). The study in the natural habitat represents a mean behaviour but the major constraints can vary this much that a precise definition of the salinity tolerance of a species (and a selection of useful plants) is not possible. These arguments open a conflict of interests: Do we need results of local importance or is it necessary to perform universally valid studies? We think a useful database for people interested in the use of cash crop halophytes should contain both information!

However as a first step, only artificial conditions in seawater 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%, 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. Former studies have shown that hydroponic cultures (soil-free) and soil cultures were not as reliable as a gravel/hydroponic system with drip (sprinkler, ditch, slack water or intertidal) irrigation (Koyro & Huchzermeyer, 1999a). Only the latter system had the potential to work under (nearly) completely artificial test conditions with high reproducibility and under close to natural conditions.

The experiments of the QCS started off at steady state conditions in a gravel/hydroponic system imitating the climatic conditions of subtropical dry regions. (Koyro & 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.

It is worth mentioning that the reliability of this cultivation system depended beside the climatic conditions (light intensity, relative humidity, air temperature)

directly on a constant periodical irrigation (15 min per 4 hrs), a sufficient O_2 supply to the roots and constant nutrient conditions (pH, nutrient composition, temperature).

The screening of potential crop halophytes (Quick-check-system, QCS) comprises the following eco-physiological tests:

1) A collection of general scientific data and some special physiological examinations of adult plants. General scientific data contain informations about factors such as germination rate, growth development, yield, reproductivity, survival (perennial species), salt induced morphological changes, photosynthesis (such as gas exchange), water relations, mineral content, content of osmotically active organic substances (such as carbohydrates and amino acids).

2) Special physiological examinations are mainly on cellular level. They include 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. However, especially in the last decade studies about gene-expression (proteomics) or genomics are getting increasingly more important.

This list is not unchangeable and can (or has to) be extended if the general and special scientific data do not allow uncovering the individual mechanisms for salinity tolerance. This variable applicable QCS seems to be valuable for the selection of useful plants and it suggest itself as a first step for the controlled establishment of cash crop halophytes because it provides detailed information about the three major goals:

1) The threshold of salinity tolerance at idealized growth conditions
2) To uncover the individual mechanisms for salt tolerance
3) The potential of utilization for the pre-selected species
   (=> cash crop halophytes).

The next chapters will demonstrate the red thread of the screening procedure and the achievement of the quick check system. Mainly, the threshold of salinity tolerance is determined as one of the first steps.

4. 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 & O'Leary, 1996). There are now reliable informations available about studies with several halophytic species from different families such as *Aster tripolium* (figure 1a), *Inula crithmoides*, *Plantago cf.*
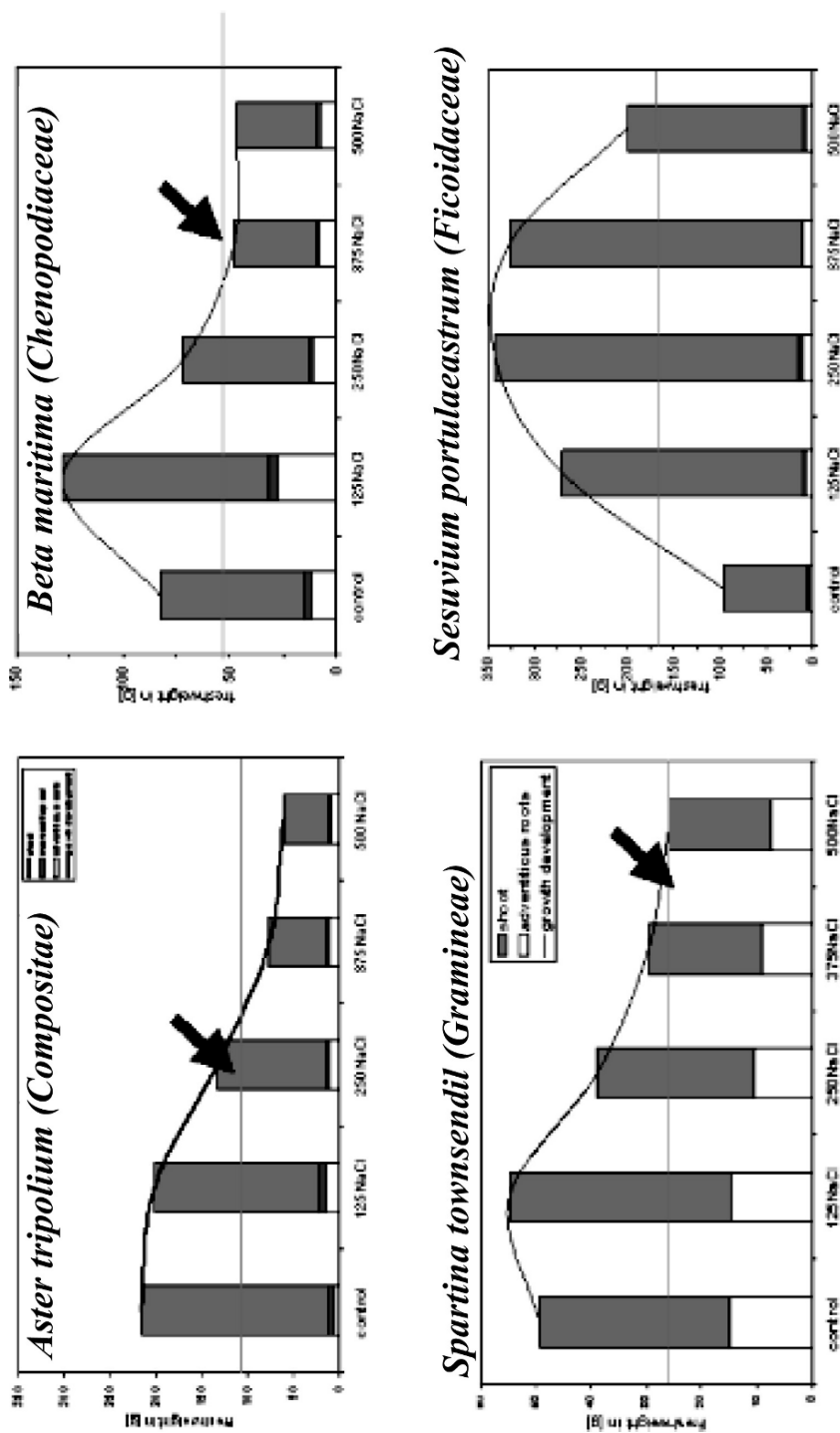
Figure 1. Threshold of salinity tolerance

coronopus, *Laguncularia racemosa, Limoneastrum articulatum*, *Beta vulgaris* ssp. *maritima* (Figure 1b), *Atriplex nummularia, Atriplex leucoclada, Atriplex halimus, Chenopodium quinoa, Batis maritima, Puccinellia maritima*, *Spartina townsendii* (Figure 1c) and *Sesuvium portulacastrum* (Figure 1d) (Pasternak, 1990; Koyro & Huchzermeyer, 1997, 1999a; Koyro et al., 1999; Lieth & Menzel, 1999; Koyro, 2000; Koyro & Huchzermeyer, 2003). The substrate-concentration leading to a growth depression of 50% (refer to freshweight, in comparison to plants without salinty) 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 (Figure 1a-d). Dramatic differences are found between halophytic plant species. The threshold of salinity tolerance amounts to 300 mol $*$ m^{-3} NaCl in *Aster tripolium*, 375 mol$*$m^{-3} in *Beta vulgaris* ssp. *maritima*, 500 mol$*$m^{-3} in *Spartina townsendii* and 750 mol$*$m^{-3} in *Sesuvium portulacastrum* (Figure 1). 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 utilization.

5. BALANCE BETWEEN WATER LOSS AND CO_2- UPTAKE

Terrestric plants at saline habitats are often surrounded by low water potentials in the soil solution and atmosphere. Plant water loss has to be minimized under these circumstances, 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 to be seen always in connection to the energy consumption and gas exchange [for example water use efficiency (WUE)]. A critical point for the plant is reached if the CO_2-fixation falls below the CO_2-production (compensation point). Therefore, one crucial aspect of the screening procedure is the study of growth reduction and net photosynthesis especially at the threshold of salinity tolerance (Figure 2).

Many plants such as *Beta vulgaris* ssp. *maritima*, *Spartina townsendii* or *Plantago* cf *coronopus* reveal at their threshold salinity tolerance a combination of low (but positive) net photosynthesis, minimum transpiration, high stomatal resistance and minimum internal CO_2-concentration (Koyro, 2000; Koyro & Huchzermeyer, 2003). However there is a big bandwidth between halophytes. Especially succulent halophytes such as *Sesuvium portulacastrum* or *Avicennia marina* have alternatives if the water balance is still positive (water uptake minus water loss) and not a 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). 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 utilization. Additionally it helps to find weak spot in the mechanisms of adjustment (of photosynthesis) to high salinity.

Sesuvium portulacastrum	A (μmol*m⁻²s⁻¹)	E (mmol*m⁻²s⁻¹)	WUE (A/E)	rs (m²*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

Aster tripolium	A (μmol*m⁻²s⁻¹)	E (mmol*m⁻²s⁻¹)	WUE (A/E)	rs (m²*s*mol⁻¹)
control	24,30	3,81	6,58	3,21
	+3,14	+0,37	+1,25	+0,29
125NaCl	17,20	3,89	4,38	5,29
	+3,69	+0,58	+0,35	+0,55
375NaCl	7,88	2,10	3,76	42,33
	+2,22	+0,63	+0,50	+2,55
500NaCl	4,26	1,22	3,49	156,74
	+0,66	+0,47	+1,68	+13,64

Figure 2. *Net photosynthesis rate [μmol * cm⁻² * s⁻¹, figure 2a and b] and water use efficiency (of the photosynthesis, [μmol CO₂ * mmol⁻¹ H₂O, figure 2a and b] of leaves of.* Aster tripolium *(a) and* Sesuvium portulacastrum *(b) at different NaCl-salinities. Many excluder-species such as* Beta vulgaris ssp. maritima *show at their threshold salt tolerance low (but positive) net photosynthesis as well as decreasing transpiration. Succulent, highly salt tolerant species show only minor changes (sometimes even an increase!) of net photosynthesis.*
0% sea water salinity = control, 25% = 125NaCl, 50% = 250NaCl, 75% = 375NaCl and 100% = 500NaCl

6. 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 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* (Sutherland & Eastwood, 1916; Walsh, 1974; Koyro & Stelzer, 1988; 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 & Breckle, 1992, 1993ab; 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; Schröder, 1998; Glaubrecht, 1999; Koyro, 2002).

7. SCREENING OF MECHANISMS TO AVOID SALT INJURY IN INDIVIDUAL SPECIES

7.1. Major constraints for plant growth on saline habitats

Many halophytic species can tolerate high sea water salinity without possessing special morphological structures (see section 6). The salinity tolerance of halophytic plants is in most cases multigenic and there is often a strong reliance between various mechanisms. It is the exception, that a single parameter is of major importance for the ability to survive at high NaCl-salinity (as already shown in section 5). 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 minimizes ion toxicity but accelerates water deficit and deminishes indirectly the CO_2-uptake. Salt absorption facilitates osmotic adjustment but can lead to toxicity and nutritional imbalance. However it should not be overlooked that beside the major constraints of high salinity less universally valid factors could be also of importance and should be kept in mind before expressing general statements:

The vegetation cycle (annual, biennial or perennial),
Ion buffer capacity of the substrate,
Duration of exposure,
Stage of plant development,
Plant organ
Environmental conditions (such as tides)

7.2. Major plant responses to high NaCl-salinity

In general the presence of soluble salts can affect growth in several ways (Mengel & 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. In principle, salinity tolerance can be achieved by salt exclusion or salt inclusion. The following physiological mechanisms to avoid salt injury (and to protect the symplast) are known as major plant responses to high NaCl-salinity (Marschner, 1995; Mengel & Kirkby, 2001; Munns, 2002; Koyro & Huchzermeyer, 2003):

(a) Adjustment of the water potential, decrease of the osmotic and matric potential, enhanced synthesis of organic solutes
(b) Regulation of the gas exchange (H_2O and CO_2), high water use efficiency (H_2O- loss per net CO_2-uptake) or/and switch to CAM-type of photosynthesis,
(c) Ion-selectivity to maintain homeostasis especially in the cytoplasm of vital organs
  - Selective uptake or exclusion (e.g. salt glands)
  - Selective ion-transport in the shoot, in storage organs, to the growing parts and to the flowering parts of the plants, retranslocation in the phloem
  - Compartmentation of Na and Cl in the vacuole
(d) High storage capacity for NaCl in the entirety of all vacuoles of a plant organ, generally in old and drying parts (e.g. in leaves supposed to be dropped later) or in special structures such as hairs. The dilution of a high NaCl content can be reached in parallel by an increase in tissue water content (and a decrease of the surface area, succulence)
(e) Avoidance of ionic imbalance
(f) Endurance of high NaCl-concentrations in the symplast
(g) Compatibility of whole plant metabolism with high NaCl-concentrations (synthesis of NaCl-tolerant enzymes, protecting agents such as proline and glycine-betaine).
(h) Restricted diffusion of NaCl in the (root-) apoplast

7.3. Benefical scientific data

Useful parameters for screening halophytes should be based 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 (see section 6, succulence, LAR: leaf mass to area ratio), growth (see section 4), water relations, gas exchange (see section 5) 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 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 & proteomics, Winicov & Bastola, 1997, 1999; Winicov, 1998).

7.4. Collection of general scientific data

Information about salt induced morphological changes; gas exchange and growth were already presented in the sections 4, 5 and 6 of this article. The list of general scientific data can be completed by information on water relations (for example leaf water potential see section 7.4.1) and composition of organic and inorganic solutes (see section 7.4.2).

7.4.1. Leaf water potential
Recognition of the importance of time frame led to the concept of a two-phase growth response to salinity (Mengel & Kirkby, 2001; Munns, 1993, 2002; Munns et al., 2002). The first phase of growth reduction is essentially a water stress or osmotic phase and presumably regulated by hormonal signals coming from the roots.

Data of the leaf water potentials (measured by dew point depression with a WESCOR HR-33T) demonstrated clearly that leaf water potential of halophytes does not correlate alone as a single factor with salinity tolerance. *Aster tripolium* (Figure 3a), *Beta vulgaris* ssp. *maritima* (Figure 3b), *Spartina townsendii* (Figure 3c), and *Sesuvium portulacastrum* (Figure 3d), have a sufficient adjustment mechanism even at high salinity treatment. The osmotic potentials were for all four halophytes (and many other) at all salinity levels sufficiently low to explain the full turgescence of the leaves (results not shown).

Assuming there is no interruption of the water supply water can flow passively from the root to the shoot and there seems to be no reason for growth reduction by water deficit for any of the studied species. However, by regulating the extent of apoplastic barriers and their chemical composition, plants can effectively regulate the uptake or loss of water and solutes (by structures such as barriers in the hypo- or exodermis). This appears to be an additional or compensatory strategy of plants to acquire water and solutes (Hose et al., 2001) and at the extremes of growth under conditions of drought and high salinity make the exodermis an absolute barrier for water and ions in the strict sense (North & Nobel, 1991; Azaizeh & Steudle, 1991) as shown for *Spartina* in figure 4.
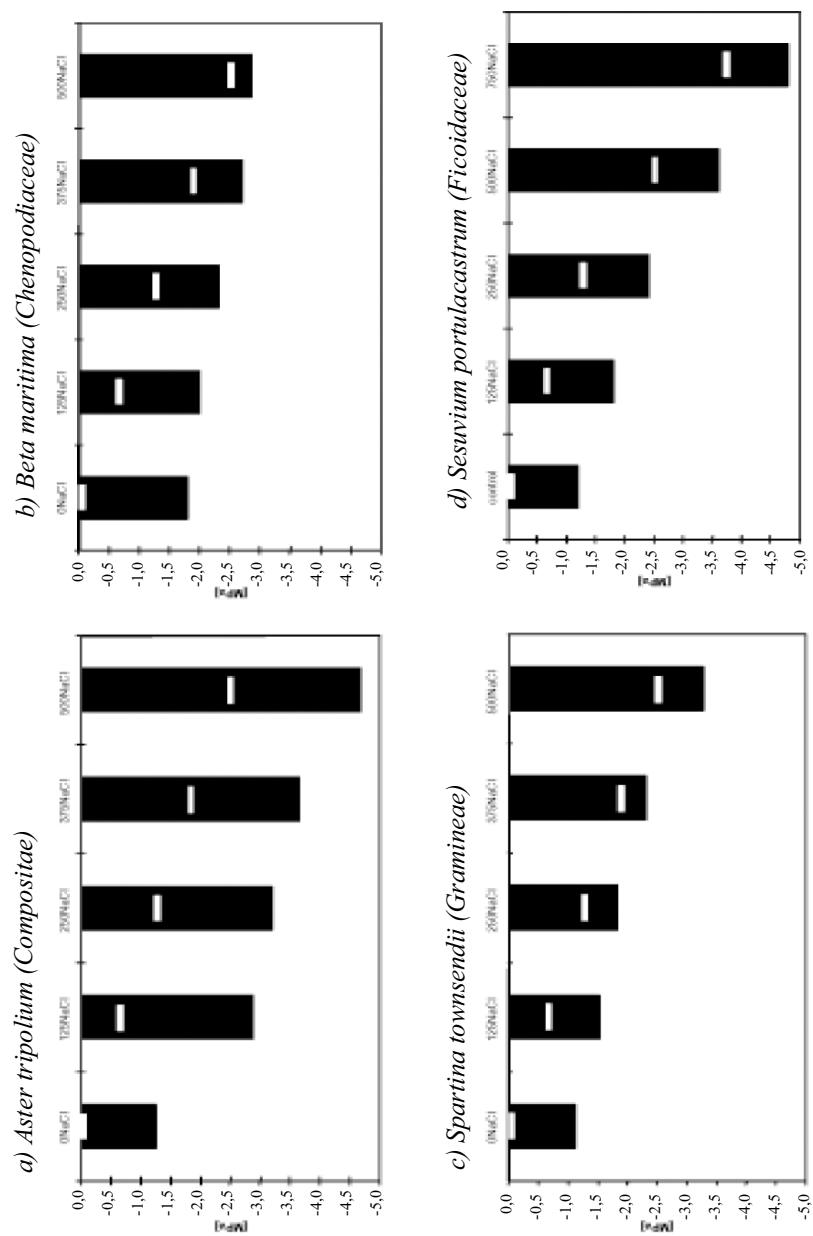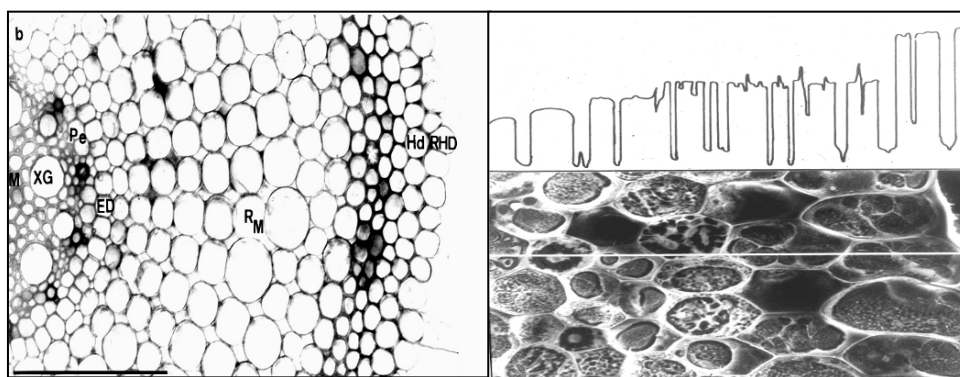
Figure 3. Leaf water potential

Figure 4. Radial cross sections in adventitious roots of Spartina townsendii.
*a) Light microscopical proof of suberin (dye Sudan III) in the cell walls of hypodermal root cells
(arrow)
b) EDXA-chlorine-specific line scans in a frozen section of a root. The white line in the electron
micrograph marks the course of the beam.*

Thus, the rate of supply of water to the shoot can be restricted due to the coupling between the flows of water and solutes (Na and Cl) even if the leaf water potential is low. Therefore, the balance between water flow (sum of water accumulation and transpiration) and the decrease in the amounts of nutrients or unfavorable nutrient ratios (e.g. Na^+/K^+) are important factors for impaired leaf elongation (Lynch et al., 1988; Munns et al., 1989) and plant growth. This is summarised a good example for the strong reliance between various mechanisms as already mentioned in section 7.1.

7.4.2. Organic and inorganic solutes

There is a second phase of growth response to salinity, which takes time to develop, and results from internal injury (Mengel & Kirkby, 2001; Munns, 1993, 2002). It is due to salts accumulating in transpiring leaves to excessive levels. Ion toxicity and nutrient imbalance are two major constraints of growth (see section 7.1) at saline habitats and therefore of special importance for the salt tolerance of halophytes. Data of additional scientific studies have shown that halophytes exhibit very different ways of adjustment to high NaCl-salinity. Generally, salt tolerant plants differ from salt-sensitive ones in having a low rate of Na^+ and Cl^- transport to leaves (Munns, 2002). However, some halophytes (so-called salt includers) even need an excess of salts for maximum growth and for attaining low solute potentials (Flowers et al., 1977; Greenway & Munns, 1980). Alternatively, high concentrations can be avoided by filtering out most of the salt. These halophytes so-called salt excluders adapt to saline conditions by ion exclusion so that osmotically active solutes have to be synthesized within the plant to meet turgor pressure demands (Mengel & Kirkby, 2001). This adaptive feature can be of importance even in species that have salt glands or bladders. However, NaCl-

Laguncularia racemosa L   *Beta vulgaris* ssp *maritima*
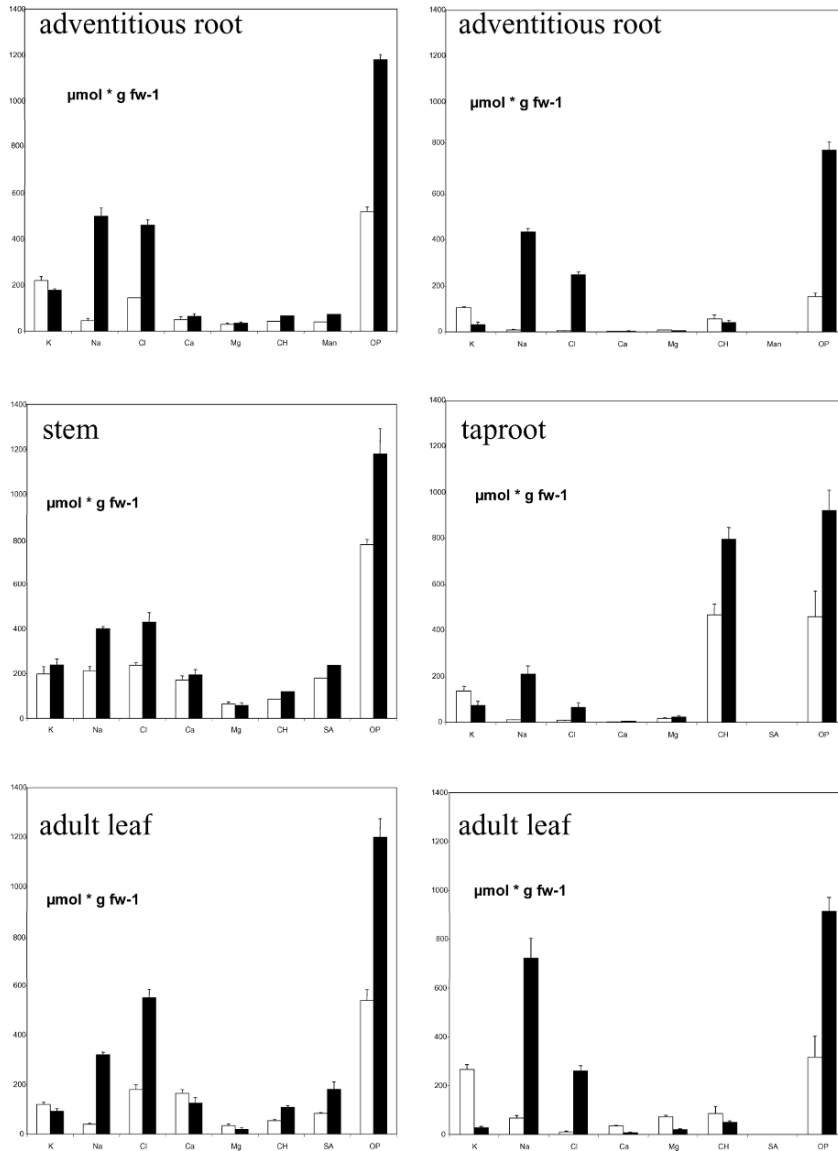
Figure 5. *Potassium-, sodium-, chlorine-, calcium, magnesium, carbohydrate and sugar alcohol-concentrations in mol * m^{-3} and osmotic values (in mOsmol) in different tissues of* Laguncularia racemosa *and* Beta vulgaris ssp. maritima.

salinity is discussed in literature mainly as if a common reaction of both ions (Na^+ and Cl^-) is leading to a salt injury. This is not always the case! For example in maize, Schubert and Läuchli (1986) did not find a positive correlation between salt tolerance and Na^+ exclusion. It is quite important to distinguish between both ions to uncover the individual mechanisms for salt tolerance.

Halophytes are able to distinguish precisely between the metabolic effects of both ions Cl^- and Na^+:

a) Some halophytes such as *Scirpus americanus*, *Avicennia marina* (with salt glands) or *Rhizophora mangle* are able to exclude Na and Cl (see literature in Kinzel, 1982) from the leaves,

b) *Laguncularia racemosa* (with salt glands) is a typical Na-excluder but with high Cl-accumulation in the leaves (figure 5a; Koyro et al., 1997),

c) *Beta vulgaris* ssp. *maritima*, *Suaeda brevifolia, Suaeda vera, Limoneastrum monopetalum, Allenrolfea occidentalis*, or *Spartina townsendii* are typical Cl-excluder with high Na-accumulation in the leaves (figure 5b; see literature in Kinzel, 1982; Koyro & Huchzermeyer, 1999a),

d) *Salicornia rubra, Salicornia utahensis, Suaeda occidentalis, Atriplex vesicaria, Atriplex nummularia, Atriplex papula*, *Atriplex rosea* or *Inula crithmoides* accumulate Na and Cl in the leaves in a range above the saline environment (salt-includers). Typical halophytic adaptation includes in this case leaf succulence in order to dilute toxic ion concentrations (Kinzel, 1982; Mengel & Kirkby, 2001).

In Na^+ and/or Cl^- excluding species (a-c), however, a lack of solutes may result in adverse effects on water balance, so that water deficiency rather than salt toxicity may be the growth-limiting factor (Greenway & Munns, 1980; Mengel & Kirkby, 2001). To achieve a low water potential and/or a charge balance the solute potential in these species is decreased by the synthesis of organic solutes (figure 5a & b) such as sugar alcohol (e.g. mannitol in leaves of *Laguncularia racemosa*; see also figure 5a), soluble carbohydrates (e.g. sucrose in taproots of *Beta maritima* ssp. *maritima*; see also Figure 5b), organic acids (incl. 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 and the formation of these solutes decreases the

energy status of the plant. Thus for plant survival, growth depression is a necessary compromise in Na^+ and /or Cl^- excluding species and not a sign of toxicity or nutrient imbalance.

7.4.3. Division of labor between leaves
The comparison between ion relations in different plant organs (root, stem, leaf) can be insufficient to uncover the individual mechanisms for salt tolerance. It can be necessary to distinguish also between different phases of growth development of one organ especially in leaves.

The comparison of the K- and Na-concentrations in juvenile and adult leaves reveals obviously that *Beta vulgaris* ssp. *maritima* uses the older parts for internal detoxification or better internal exclusion of sodium (Koyro, 2000). The steep inverse Na/K gradient between juvenile and adult leaves of the sea beet is a typical reaction of many halophytes to high NaCl-salinity (Wolf et al., 1991; Koyro & Huchzermeyer, 1999a). There is scientific debate whether high potassium concentrations in young leaves and reproductive organs can be achieved by low xylem import of both potassium and sodium, and/or high phloem imports from mature leaves (Wolf et al., 1991).

The chlorine just like the sodium concentrations was much higher in adult than in juvenile leaves. An effective restriction of sodium and chlorine import into young leaves as compared to old leaves is also typical for *Agrostis stolonifera* (Robertson & Wainwright, 1987). Another mechanism of sea beet to reduce internal NaCl-concentrations was shedding of old leaves (see literature in Koyro, 2000). This system successfully deminishes and controls the accumulation of Na and Cl in younger or metabolically active parts of the plant and buffers against an imbalance of nutrients such as K.

7.5. Special physiological examination

The general scientific data give an impression of various mechanisms of adaptation to high NaCl-salinity. Beside water stress and ion specific toxic physiological disorders on tissue level (see section 7.4.1 and 7.4.2) intracellular ionic imbalances (K^+, Ca^{2+} and Mg^{2+}) can be caused by high salt concentrations (Mengel & 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. However, the analysis of the distribution of elements (compartmentation) in a distinct cell type can be used to answer several questions such as:

(a) Is there a compartmentation and a division of labour in cytoplasm and vacuole?
(b) Is it necessary to look more in detail to toxic effects caused by high cytoplasmic sodium or chlorine concentrations?
(c) Is there a compatibility of the metabolism with high NaCl-concentrations in the symplast (cytoplasm)?
(d) Is there a high ionic imballance in the symplast (cytoplasma)?

Beta vulgaris ssp. *maritima* and *Spartina townsendii* are typical Cl-excluder with high Na-accumulation in the leaves (see section 7.4.1). Both species seem to react similar to salinity with changes of leaf water potential, gas-exchange and

nutrients. However, this number of congruence 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 mature leaves (Mengel & Kirkby, 2001). This is because 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 & Huchzermeyer, 2003). These changes can be interpreted as signs of a critical load. Therefore, to distingiuish 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 cytolasmatic composition in cells of the upper leaf-epidermis are summarized for the controls and the high-salinity treatments (at seawater salinity) in figure 6. 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 contains 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 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 & Polard, 1983; Koyro & Stelzer, 1988).

It is obvious that seawater 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. 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 hypothesized 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 & Kirkby, 2001). One possible consequence is the supply of sufficient fertilizers (especially K and P) at high NaCl-salinity to reduce the symptoms of K- and P-deficiency in Beta.

Beta vulgaris ssp. *maritima*

adaxial	vacuole				cytoplasm			
leafepidermis	control		480 NaCl		control		480 NaCl	
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

adaxial	vacuole				cytoplasm			
leafepidermis	control		480 NaCl		control		480 NaCl	
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	±2.06	<5		<5	

Figure 6. *Sodium- magnesium-, phosphor-, sulphur-, chlorine- and potassium-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.

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 lead to the long-term growth differences between the salt-tolerant and 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.

7.6. Evaluation of the screening procedure

The results presented in this paper 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.

8. SELECTION OF SALINITY TOLERANT SPECIES WITH PROMISING TOLERANCE AND YIELD CHARACTERISTICS

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 paper. Four steps are prerequisites for the selection of appropriate salinity tolerant plants with promising tolerance (and yield) characteristics.

(a) 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.

(b) 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 responses have to be evaluated for precise informations of ecophysiological demands. The data can build up a well-founded basis for the improvement of the utilisation potential.

9. 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). However, it can be only the first step for the development of cash crops or other usable plants from existing halophytes. After the selection of halophytic species suited for a particular climate and for a particular utilisation a gradual realization of the following topics could be one way to establish potentially useful cash crop halophytes:

(a) Green house experiments at the local substrates (and climatic conditions) to select and propagate promising sites (Isla et al., 1997).

(b) Studies with Lysimeters on field site to study the water consumption and ion movements.

(c) Design of a sustainable production system in plantations at coastal areas or at inland sites (for example for economical use).

(d) Testing yield and (economic) acceptance of the product.

10. REFERENCES

Abdelly, C., Lachaal, M. & Grignon, C. 1999. Importance of micro-heterogenity of salinity and fertility for maintenance of the plant diversity. In: H. Lieth, M. Moschenko, M. Lohmann, H. W. Koyro & A. Hamdy (Eds.), Progress in Biometeorology. Leiden, Netherlands: Backhuys Publishers, 65-76 pp.

Amzallag, G.N. 1994. Influence of parental NaCl treatment on salinity tolerance of offspring in *Sorghum bicolor* (L) Moench. New Phytologist 128: 715-723.

Ashraf, M. & O'Leary, J.W. 1996. Effect of drought stress on growth, water relations, and gas exchange of two lines of sunflower differing in degree of salt tolerance. International Journal of Plant Sciences 157: 729-732.

Azaizeh, H. & Steudle, E. 1991. Effects of salinity on water transport of excised maize (*Zea mays* L.) roots. Plant Physiology 97: 1136-1145.

Boer, B. & Gliddon, D. 1998. Mapping of coastal ecosystems and halophytes (case study of Abu Dhabi, United Arab Emirates). Marine and Freshwater research 49: 297-301.

Ellenberg, H. 1974. Zeigerwerte der Gefäßpflanzen Mitteleuropas. Scripta Geobotanica 9: 97.

Flowers, T.J., Troke, P.F. & Yeo, A.R. 1977. The mechanisms of salt tolerance in halophytes. Annual Review Plant Physiology 28: 89-121.

Freitas, H. & Breckle, S.W. 1992. Importance of bladder hairs for salt tolerance of field-grown *Atriplex*-species from a Portuguese salt marsh. Flora 18: 283-297.

Freitas, H. & Breckle, S.W. 1993a. Progressive cutinization in *Atriplex* bladder stalk cells. Flora 188: 287-290.

Freitas, H. & Breckle, S.W. 1993b. Accumulaton of nitrate in bladder hairs of *Atriplex* species. Plant Physiological Biochemistry 31: 887-892.

Ghassemi, F., Jakeman, A.J. & Nix, H.A. 1995. Salinisation of land and water resources: Human causes, extent, management and case studies. Sydney, Australia: USNW Press. 540 pp.

Glaubrecht, M. 1999. Mangrove der tropischen Gezeitenwälder; Naturw. Rdsch 52.

Greenway, H. & Munns, R. 1980. Mechanisms of salt tolerance in nonhalophytes. Annal Review Plant Physiology 31: 149-190.

Hose, E., Clarkson, D.T., Steudle, E., Schreiber, l. & Hartung, W. 2001. The exodermis: a variable apoplastic barrier. Journal of Experimental Botany 52: 2245-2264.

Igartua, E. 1995. Choice of selection environment for improving crop yields in saline areas. Theoretical and Applied Science 91: 1016-1021.

Jeschke, W.D., Klagges, S., Hilpert, A., Bhatti, A.S. & Sarwar, G. 1995. Partitioning and flows of ions and nutrients in salt-treated plants of *Leptochloa fusca* L Kunth .1. Cations and chloride. New Phytologist 130: 23-35.

Isla, R., Royo, A. & Aragues, R. 1997. Field screening of barley cultivars to soil salinity using a sprinkler and a drip irrigation. Plant and Soil 197: 105-117.

Kinzel, H. 1982. Pflanzenökologie und Mineralstoffwechsel. Stuttgart, Germany: Eugen Ulmer Publisher.

Koyro, H.-W. & Stelzer, R. 1988. Ion concentrations in the cytoplasm and vacuoles of rhizodermal cells from NaCl treated Sorghum, *Spartina* and *Puccinellia* plants. Journal of Plant Physiology 133: 441-446.

Koyro, H.-W. & Lieth, H. 1998. Salinity conversion table. 2nd enlarged Edition, © H.Lieth ISSN 09336-3114, Osnabrück.

Koyro, H.-W. & Huchzermeyer, B. 1997. The physiological response of *Beta vulgaris* ssp. *maritima* to seawater irrigation. In: H. Lieth, A. Hamdy & H.-W. Koyro, (Eds.), Water management, salinity and pollution control towards sustainable irrigation in the mediterranean region. Salinity problems and halophyte use. Bari, Italy: Tecnomack Publications. 29-50 pp.

Koyro, H.-W., Wegmann, L., Lehmann, H. & Lieth, H. 1997. Physiological mechanisms and morphological adaptation of *Laguncularia racemosa* to high salinity. In: H. Lieth, A. Hamdy & H.-W. Koyro, (Eds.), Water management, salinity and pollution control towards sustainable irrigation in the mediterranean region: Salinity problems and halophyte use. Bari, Italy: Tecnomack Publications, 51-78 pp.

Koyro, H.-W. & Huchzermeyer, B. 1999a. Influence of high NaCl-salinity on growth, water and osmotic relations of the halophyte *Beta vulgaris* ssp. *maritima*. Development of a quick check In: H. Lieth, M. Moschenko, M. Lohmann, H.-W. Koyro & A. Hamdy, (Eds.), Progress in Biometeorology, Leiden, Netherlands: Backhuys Publishers. 87-101 pp.

Koyro, H.-W. & Huchzermeyer, B. 1999b. Salt and drought stress effects on metabolic regulation in maize. In: M. Pessarakli, (Ed.), Handbook of plant and crop stress 2nd Ed. New York, New York: Marcel Dekker Inc. 843-878 pp.

Koyro, H.-W., Wegmann, L., Lehmann, H. & Lieth, H. 1999. Adaptation of the mangrove Laguncularia racemosa to high NaCl salinity. In: H. Lieth, M. Moschenko, M. Lohmann, H.-W Koyro, & A. Hamdy. (Eds.) Progress in Biometeorology, Leiden, Netherlands: Backhuys Publishers. 41-62 pp.

Koyro, H.-W. 2000. Untersuchungen zur Anpassung der Wildrübe (*Beta vulgaris* ssp. *maritima*) an Trockenstreß oder NaCl-Salinität. Habilitation. Giessen, Germany: Justus-Liebig-University.

Koyro, H.-W. 2002. Ultrastructural effects of salinity in higher plants. In: A. Läuchli & U. Lüttge. (Eds.), Salinity: Environment – Plants – Molecules. Dordrecht, Netherlands: Kluwer Academic Publication. 139‑158 pp.

Koyro, H.-W. & Huchzermeyer, B. 2003. Ecophysiological needs of the potential biomass crop Spartina townsendii Grov.. Journal of Tropical Ecology, (in press).

Landolt, E. 1977. Ökologsche Zeigerwerte zur Schweizer Flora. Veröffentlichungen des geobotanischen Instituts der Eidgenössischen Technischen Hochschule in Zürich 64, Stiftung Ruebel, 208 pp.

Läuchli, A. 1999. Potassium interactions in crop plants. In: D.M. Oosterhuis, & G.A. Berkowitz. (Eds.), Frontiers in Potassium Nutrition. New perspectives on the effects of potassium on physiology of plants. New York, New York: Marcel Dekker. 71-76 pp.

Lieth, H. 1999. Development of crops and other useful plants from halophytes, In: H. Lieth, M. Moschenko, M. Lohmann, H.-W Koyro, & A. Hamdy (Eds.), Halophytes Uses in different Climates, Ecological and Ecophysiological Studies. Leiden, Netherlands:Backhuys Publishers, 1-18 pp.

Lieth, U. & Menzel, U. 1999. Halophyte Database Vers. 2, In: H. Lieth, M. Moschenko, M. Lohmann, H.-W Koyro, & A. Hamdy. (Eds.), Halophytes Uses in different Climates, Ecological and Ecophysiological Studies, Leiden, Netherlands:Backhuys Publishers. 159-258 pp.

Lieth, H., Moschenko, M., Lohmann, M., Koyro, H.-W. & Hamdy, A. 1999. Halophyte uses in different climates I. Ecological and ecophysiological studies. In: H. Lieth, (Ed.), Progress in Biometeoroogy, Leiden, Netherlands:Backhuys Publishers. 1-258 pp.

Lynch, J., Thiel, G. & Läuchli, A. 1988. Effects of salinity on the extensibility and Ca availability in the expanding region of growing barley leaves. Botanica Acta 101: 355-361.

Marcum, K.B., Anderson, S.J. & Engelke, M.C. 1998. Salt gland ion secretion: A salinity tolerance mechanism among five zoysiagrass species. Crop Science 38: 806-810.

Marcum, K.B. 1999. Salinity tolerance mechanisms of grasses in the subfamily Chloridoideae. Crop Science 39: 1153-1160.

Marschner, H. 1995. Mineral nutrition of higher plants. New York, New York: Academic Press. 1-889 pp.

Mengel, K. & Kirkby, E.A. 2001, Principles of Plant Nutrition. Dordrecht, Netherlands: Kluwer Academic Publisher. 1-849 pp.

Munns, R., Gardner, P.A., Tonnet, M.L. & Rawson, H.M. 1989. Growth and development in NaCl-treated plants. II Do Na+ or Cl- concentrations in deviding or expanding tissues determine growth in barley. Australian Journal Plant Physiology 15: 529-540.

Munns, R. 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell and Environment 16: 15-24.

Munns, R. 2002. Comparative physiology of salt and water stress. Plant Cell and Environment 25: 239-250.

Munns, R., Husain, S., Rivelli, A.R., James, R.A., Condon, A.G., Lindsay, M.P., Lagudah, E.S., Schachtman, D.P. & Hare, R.A. 2002. Avenues for increasing salt tolerance of crops, and the role of physiologically based selection traits. Plant and Soil 247: 93-105.

North, G.B. & Nobel, P.S. 1991. Changes in hydraulic conductivity and anatomy caused by drying and rewetting roots of Agave desertii, Agavaceae. American Journal of Botany 78: 906-915.

Pasternak, D. 1990. Fodder production with saline water. The institute for applied research, Beer-Sheva/Israel: Ben Gurion University of the Negev. 173 pp.

Robertson, K.P. & Wainwright, J.J. 1987. Photosynthetic responses to salinity in two clones of Agrostis stolonifera. Plant Cell and Environment 10: 45-52.

Schimper, A.F.W. 1891. Pflanzengeographie auf physiologischer Grundlage. Jena:Fischer Publication.

Schroeder, F.G. 1998. Lehrbuch der Pflanzengeographie; Wiesbaden: Quelle & Meyer.

Schubert, A. & Läuchli, A. 1986. Na$^+$ exclusion, H$^+$ release and growth of two different maize cultivars under NaCl salinity. Journal Plant Physiology 61: 145-154.

Sutherland, G.K. & Eastwood, A. 1916. The physiological anatomy of *Spartina townsendii.* Annuals Botany 30: 333-351.

Szabolcs, I. 1994. Soils and salinisation. In: M. Pessarakli. (Ed.), Handbook of Plant and Crop Stress. New York: Marcel Dekker. 3-11 pp.

U.S. Salinity Laboratory Staff 1954 Diagnosis and improvement of saline and alkali soils. In: L. A. Richards. (Ed.). Agricultural Handbook of the U.S. Department of Agriculture, Washington D.C.:Government Printing. 157 pp.

Tazuke, A. 1997. Growth of cucumber fruit as affected by the addition of NaCl to nutrient solution. Journal of the Japanese Society for Horticultural Science 66: 519-526.

Volkmar, K.M., Hu, Y. & Steppuhn, H. 1998. Physiological responses of plants to salinity: A review. Canadian Journal of Plant Science 78: 19-27.

Walsh, G.E. 1974. Mangroves. A review. In: R. J. Reimold, & W. H. Queen. (Eds.), Ecology of halophytes. New York, New York: Academic Press. 51-174 pp.

Warne, T.R., Hickok, L.G., Sams, C.E. & Vogelien, D.L. 1999. Sodium/potassium selectivity and pleiotropy in stl2, a highly salt-tolerant mutation of *Ceratopteris richardii*. Plant Cell and Environment 22: 1027-1034.

Weber, E. & D'Antonio, C.M. 1999. Germination and growth responses of hybridizing *Carpobrotus* species (Aizoaceae) from coastal California to soil salinity. American Journal of Botany 86: 1257-1263.

Winicov, I. & Bastola, D.R. 1997. Salt tolerance in crop plants: New approaches through tissue culture and gene regulation. Acta Physiologiae Plantarum 19: 435-449.

Winicov, I. 1998. New molecular approaches to improving salt tolerance in crop plants. Annals of Botany 82: 703-710.

Winicov, I. & Bastola, D.R. 1999. Transgenic overexpression of the transcription factor Alfin1 enhances expression of the endogenous MsPRP2 gene in alfalfa and improves salinity tolerance of the plants. Plant Physiology 120: 473-480.

Winter, U., Kirst, G.O., Grabowski, V., Heinemann, U., Plettner, I. & Wiese, S. 1999. Salinity tolerance in *Nitellopsis obtusa*. Australian Journal of Botany 47: 337-346.

Wolf, O., Munns, R., Tonnet, M.L. & Jeschke, W.D. 1991. The role of the stem in the partitioning of Na+ and K+ in salt treated barley. Journal of Experimental Botany 42: 697-704.

Wyn Jones, R.G., Brady, C.J. & Speirs, J. 1979. Ionic and osmotic relations in plant cells. In: D.L. Laidman, & R.G. Wyn Jones. (Eds.), Recent Advances in the Biochemistry of Cereals. New York, New York: Academic Press. 1-391 pp.

Wyn Jones, R.G. & Pollard, A. 1983. Proteins, enzymes and inorganic ions. In: A. Läuchli, & R. L. Bieleski. (Eds.). Inorganic Plant Nutrition. Encyclopedia of Plant Physiology 15b, Hiedelberg, Germany: Springer Verlag. 528-555 pp.

Yeo, A. 1998. Molecular biology of salt tolerance in the context of whole-plant physiology. Journal of Experimental Botany 49: 915-929.