

## Strategies of a potential cash crop halophyte (*Beta vulgaris* ssp. *maritima*) to avoid salt injury

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**Abstract:** Salt stress effects were studied on *Beta vulgaris* ssp. *maritima* plants cultivated in defined nutrient solution in a gravel/hydroponic quick check system (QCS) with flow irrigation. We compared plants grown with 0, 125, 250, 375 and 500 mol m<sup>-3</sup> NaCl-salinity. The salinity tolerance was measured on the basis of three parameters: a) The maximum yield of *Beta vulgaris* ssp. *maritima* was reduced by 50% (C<sub>50</sub>-value) at 375 mol m<sup>-3</sup> NaCl-salinity. b) The salinity threshold was reached at 125 mol m<sup>-3</sup> NaCl-salinity. c) Gas exchange parameters such as net photosynthesis (μmol m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance (mol m<sup>-2</sup> s<sup>-1</sup>) and transpiration (mol m<sup>-2</sup> s<sup>-1</sup>) were highest at 250 mol m<sup>-3</sup> NaCl-salinity. At salinities above the C<sub>50</sub>-value the ratio of intercellular (C<sub>i</sub>) to atmospheric (C<sub>a</sub>) CO<sub>2</sub>-concentrations (C<sub>i</sub>/C<sub>a</sub> ratios), transpiration and stomatal conductance showed a distinct reduction and there was a steep rise in water use efficiency. The strategies employed by the seabeet for avoiding salt injury depend on adaptation to low water potential and high Na and Cl concentrations. The seabeet was able to balance out low external water potential and to generate turgor by accumulating high internal Na and Cl concentrations in the leaf and of sucrose and proline in the taproot. However, the specificity for the uptake of K, Mg and Ca was insufficient to prevent dilution of these ions at tissue and cellular level, supporting the hypothesis that the major reason for the particular threshold of salinity tolerance in the seabeet is ion-deficiency. The biennial seabeet survives NaCl in the range of seawater salinity because of a division of labour between leaf and taproot. It can be concluded that the mechanisms in the leaf are effective only until the taproot provides sufficient conditions for completion of a second vegetation period.

**Resumen:** Se estudiaron los efectos del estrés causado por la sal en plantas de *Beta vulgaris* ssp. *maritima* cultivadas en una solución nutritiva definida, en un sistema hidropónico/grava de rápida revisión (QCS) con irrigación de flujo. Comparamos plantas que crecieron con salinidades de NaCl de 0, 125, 250, 375 y 500 mol m<sup>-3</sup>. La tolerancia a la salinidad fue medida con base en tres parámetros: a) El máximo rendimiento de *Beta vulgaris* ssp. *maritima* se vio reducido en 50% (valor de C<sub>50</sub>) en una salinidad de NaCl de 375 mol m<sup>-3</sup>. b) El umbral de salinidad fue alcanzado en una salinidad de NaCl de 125 mol m<sup>-3</sup>. c) Los parámetros de intercambio de gases tales como la fotosíntesis neta (μmol m<sup>-2</sup> s<sup>-1</sup>), la conductancia estomática (mol m<sup>-2</sup> s<sup>-1</sup>) y la transpiración (mol m<sup>-2</sup> s<sup>-1</sup>) tuvieron sus máximos en una salinidad de NaCl de 250 mol m<sup>-3</sup>. En salinidades por arriba del valor de C<sub>50</sub>, el cociente de concentraciones de CO<sub>2</sub> intercelular (C<sub>i</sub>) sobre el atmosférico (C<sub>a</sub>) (cociente C<sub>i</sub>/C<sub>a</sub>), la transpiración y la conductancia estomática mostraron una reducción distinta y hubo un

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aumento muy abrupto en la eficiencia de uso del agua. Las estrategias empleadas por la remolacha de mar para evitar daños por salinidad dependen de la adaptación a un bajo potencial hídrico y a concentraciones altas de Na y Cl. La remolacha de mar fue capaz de balancear un bajo potencial hídrico externo y de generar turgencia por medio de la acumulación de concentraciones internas altas de Na y Cl en la hoja, y de sacarosa y prolina en la raíz pivotante. Sin embargo, la especificidad de la absorción de K, Mg y Ca fue insuficiente para prevenir la dilución de estos iones a nivel tisular y celular, lo que apoya la hipótesis de que la causa principal del umbral particular de tolerancia a la salinidad en la remolacha de mar es la deficiencia de iones. La remolacha bienal sobrevive al NaCl en el intervalo de salinidad del agua de mar gracias a una división del trabajo entre la hoja y la raíz pivotante. Se puede concluir que el mecanismo en la hoja es efectivo sólo si la raíz pivotante proporciona condiciones suficientes para poder completar un segundo periodo de crecimiento vegetativo.

**Resumo:** Os efeitos do stress salino foram estudados em plantas de *Beta vulgaris* ssp. *maritima* cultivadas numa solução nutriente definida num sistema de verificação rápida do tipo hidropónica/cascalho (QCS) com fluxo de irrigação. O crescimento das plantas foi comparado com salinidades de 0,125, 250, 375 e 500 mol m<sup>-3</sup>. A tolerância à salinidade foi medida na base de três parâmetros: a) O rendimento máximo da *Beta vulgaris* ssp. *maritima* foi reduzido a 50% (valor C<sub>50</sub>) para a salinidade de 375 mol m<sup>-3</sup> NaCl; b) O limiar de salinidade foi atingido a 125 mol m<sup>-3</sup> NaCl; c) Os parâmetros de troca gasosa tais como a fotossíntese líquida (μmol m<sup>-2</sup> s<sup>-1</sup>), a condutância estomática (mol m<sup>-2</sup> s<sup>-1</sup>) e a transpiração (mol m<sup>-2</sup> s<sup>-1</sup>) foram mais elevadas para uma salinidade de 250 mol m<sup>-3</sup> NaCl. Para valores da salinidade acima do valor de C<sub>50</sub> o ratio (C<sub>i</sub>/C<sub>a</sub>), traduzido pela relação entre o valores das concentrações da concentração do CO<sub>2</sub> intercelulares (C<sub>i</sub>) e atmosférico (C<sub>a</sub>), a transpiração e condutância estomática mostraram uma redução clara e houve uma aumento brusco na eficiência no uso da água. As estratégias empregues pela beterraba marítima para evitar as injúrias provocadas pelo sal depende da adaptação ao baixo potencial hídrico a altas concentrações de Na e Cl. A beterraba marítima foi capaz de compensar o baixo potencial hídrico externo e gerar turgescência mediante uma acumulação interna da Na e Cl elevada na folha e de sacarose e prolina na raiz principal. Contudo, a especificidade para a absorção do K, Mg e Ca foi insuficiente para prevenir a diluição destes iões ao nível dos tecidos e celular, suportando a hipótese de que a maior razão para o limiar particular de tolerância da beterraba marítima à salinidade seja a deficiência iónica. A beterraba marítima bienal sobrevive ao NaCl no intervalo de salinidade da água do mar por causa da divisão de trabalho entre a folha e a raiz principal. Pode concluir-se que o mecanismo na folha só é efectivo enquanto a raiz principal proporcionar condições suficientes para completar o segundo período vegetativo.

**Key words :** Halophyte, *Beta vulgaris*, glycophytes.

## Introduction

The sustainable use of halophytic plants is a promising approach to valorizing strongly salinised zones unsuitable for conventional agriculture and exhibiting mediocre water quality (Lieth *et al.* 1999). There are already many halophytic species that are exploited for purposes economic (human food, fodder) or ecological (soil desalinisation, dune

fixation, CO<sub>2</sub>-sequestration). However, the broad potential for halophyte utilisation has scarcely begun to be explored. Halophytes are plants that are able to complete their life cycle in a substrate rich in NaCl. One of the most important properties of halophytes is their salinity tolerance (Lieth *et al.* 1999). This substrate gives obligate halophytes decided advantages over salt-sensitive plants (glycophytes). There is a wide range of tolerance

among the 2,600 known halophytes (Lieth & Menzel 1999; Pasternak 1990). However, information about these halophytes need careful checking for reliability. A precondition for a sustainable utilisation of suitable halophytes is precise knowledge of their salinity tolerance and the various mechanisms that enable a plant to grow in its natural saline habitat (Winter *et al.* 1999).

It is necessary to develop sustainable biological production systems which can tolerate higher water salinity, in view of the fact that freshwater resources will become limited in the near future (Lieth *et al.* 1999). Potentially useful halophytes can be studied under reproducible experimental growth and substrate conditions only in the artificial environment of a photoperiodically controlled growth cabinet mimicking sea-water irrigation systems. The supply of different degrees of sea-water salinity (0%, 25%, 50%, 75%, 100%, and, if necessary, supersaturation) to the roots in separate systems under otherwise identical conditions ensures the necessary preconditions for a rapid-checking system (QCS) for promising cash-crop halophytes (Koyro & Huchzermeyer 2004). This QCS would seem to be ideal for the selection of useful plants and it suggests itself as a first step in the controlled establishment of cash-crop halophytes, providing as it does detailed information about the following three major criteria: 1. the threshold of salinity tolerance (for a definition, see the section on Material and methods); 2. determining the individual mechanisms involved in salinity tolerance; and 3. potential utilization of the pre-selected species.

In accordance with the definition of salinity tolerance, growth parameters (such as the salinity threshold and the the  $C_{50}$ -value) and gas exchange parameters [such as net photosynthesis, intercellular ( $C_i$ ) and atmospheric ( $C_a$ )  $CO_2$ -concentrations,  $C_i/C_a$  ratios, stomatal conductance, and water use efficiency measured on the youngest fully emerged leaf blades] are used as objective parameters for the description of the actual condition of a plant (Kinzel 1982; Shannon & Grieve 1999). Reliable information is now available on studies of several species such as *Plantago* cf. *coronopus*, *Laguncularia racemosa*, *Aster maritima*, *Batis maritima* and *Spartina townsendii* (Koyro 2000; Koyro *et al.* 1999; Koyro & Huchzermeyer 2004).

Terrestrial plants in saline habitats are often surrounded by low water potential 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 maintain high net photosynthesis at low water loss rates. In this context, the biomass production of a plant has always to be seen in connection with 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 level of  $CO_2$ -production (compensation point). One crucial parameter of the QCS is, therefore, the study of growth reduction and net photosynthesis dependency, especially at the threshold of salinity tolerance. In principle, salinity tolerance can be achieved by salt exclusion or salt inclusion. Several physiological mechanisms for avoiding salt injury (and protecting the symplast) are described as major plant responses to high NaCl-salinity (Koyro & Huchzermeyer 2004; Mengel & Kirkby 2001).

The goals of this research is to get a survey of the mechanisms leading to the salinity tolerance of this species. It will be shown that the salinity-induced multiplicity of functional and anatomical changes constitutes a group of indicators for the salinity tolerance and growth potential of this species. These indicators and plant specific factors such as plant development or division of labour between plant tissues, cells and cell compartments provide a reliable, ecophysiological basis as the first step for the development of a ecological sustainable and economically feasible halophytic cash crop.

## Material and methods

### *Plant growth*

Seeds of *Beta vulgaris* ssp. *maritima* were washed for several minutes with warm tap water and imbibed for one day in the dark (25 °C) in an aerated 0.2 mol m<sup>-3</sup> CaSO<sub>4</sub>-solution. The seeds were then transferred to a Petri dish with wet filter paper (0.2 mol m<sup>-3</sup> CaSO<sub>4</sub>) for germination in a dark growth cabinet at 25°C. Seven to 14 days after germination, plants were potted into soil (type LD 80, Fa Archut, Lawkbach, Germany). After a further 2 weeks the young seedlings were transplanted into a soil-less (gravel/hydroponics) culture quick-check system (Koyro 2000). The

plants were irrigated with a basic nutrient solution as modified by Epstein (1972) under photoperiodic conditions (16 h light/ 8 h dark) in an environmentally controlled greenhouse (Koyro 2000). Temperatures were  $25 \pm 2$  °C during the day and  $15 \pm 2$  °C during the night. Relative humidity ranged from 45% to 70%. Light intensity was in the range of  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  at plant level.

The stepwise addition of NaCl to the basic nutrient solution began after a period of another 2 weeks by raising the salinity of the solution in steps of  $50 \text{ mol m}^{-3}$  NaCl each day. There were five treatments altogether: control, 125, 250, 375, and  $500 \text{ mol m}^{-3}$  NaCl (equivalent to 0, 25, 50, 75 and 100 % NaCl). The highest salinity treatment was reached after 9 days. The quick-check system was programmed by a timer to water the plants every 4 h for 30 minutes starting at midnight, 4 a.m., 8 a.m., 12 noon, 16 p.m. and 20 p.m. daily and to allow the saline solutions to drain freely from the pots. Solutions were recycled and changed every 2 weeks to avoid nutrient depletion. The experiment was performed for a total period of 6 weeks.

#### *Yield threshold, salinity tolerance and quantitative chemical analysis*

The number and succulence (defined as weight per surface area) of the leaves and the weight of plants, leaves and roots (main and adventitious roots) were noted. Salinity tolerance was measured satisfactorily on the basis of two growth-related parameters (Shannon & Grieve 1999): a). The threshold (t) is the NaCl-salinity that is expected to cause an initial significant reduction in the maximum expected yield ( $Y_{\text{max}}$ ). b). The numerically most reliable value for crop salinity tolerance response studies is the  $C_{50}$ -value (maximum yield is reduced by 50%, Kinzel 1982).

The plant was divided into two parts (below and above ground) for quantitative chemical analysis and washed separately for 1 minute in ice-cooled  $0.2 \text{ mol m}^{-3}$   $\text{CaSO}_4$  solution, 1 minute in aqua dest., and blotted carefully with tissue paper. Different parts of the root system (tap root and adventitious roots) and juvenile and adult leaves (divided into lamina and petiole) were weighed and extracted with 0.5%  $\text{HNO}_3$  in a water bath (80°C) for 12 h. Na and K were determined from the extract with an atomic absorption spectrophotometer (Perkin Elmer PE3300) and Cl

by electrochemical titration (AMINCO COTLOVE chloride titration).

#### *CO<sub>2</sub>-gas exchange and water potential*

Gas exchange parameters of the leaves were measured to obtain further information about the salinity tolerance of the seabiet and especially about the water use efficiency at higher NaCl-salinities. The closed photosynthesis measurement system LI-COR 6200 (LI-COR, Lincoln, NE, USA) was used to determine the response of photosynthesis to substrate salinity. An initial concentration of  $400 \pm 14$  ppm was adjusted by using a gas-tight Hamilton injection filled with pure  $\text{CO}_2$ . Photosynthesis reduced the concentration in the closed loop until the compensation point was reached (Grüters *et al.* 2000). Calculation of the gas exchange parameters was corrected for leaks, as described by LI-COR Inc. Net photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), intercellular ( $C_i$ ) and atmospheric ( $C_a$ )  $\text{CO}_2$ -concentrations (ppm),  $C_i/C_a$  ratios, stomatal conductance ( $\text{mol m}^{-2} \text{s}^{-1}$ ), transpiration ( $\text{mol m}^{-2} \text{s}^{-1}$ ) and water-use efficiency ( $\mu\text{mol mmol}^{-1}$ ) were measured on the youngest fully emerged leaf blades. All measurements were taken at light saturation ( $Q = 1650 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and 24-26 °C.

The leaf water potential was measured psychrometrically on the abaxial surface of intact plants with a Dew Point Microvoltmeter (Wescor Type HR 330, WESCOR INC. USA).

#### *Carbohydrate analysis*

Qualitative and quantitative analyses of soluble carbohydrates in hydrous extracts of freeze-dried samples was performed by high-performance anion exchange (HPAE) with pulsed amperometric detection (Hoffmann-Thoma *et al.* 2001). Separation of sugars was carried out on a CarboPac PA-100 column with a CarboPac PA 100 guard column (Dionex, Sunnyvale, Calif., USA).

#### *X-ray microanalyses (EDXA)*

Leaf sections (15 sections per treatment) were mounted on a Cu-specimen stub together with droplets of standard solutions. The sections were cut transversely with a blade cooled with liquid nitrogen on a cryo-stage under high vacuum conditions, then transferred to the column of an ETEC Autoscan SEM or a Philips XL20 equipped

with a KEVEX Si/Li detector for X-ray count harvesting. The electron beam was focussed on cytoplasm and vacuoles of epidermal cells and on vacuoles of palisade and spongy parenchym cells. Subsequent quantification procedures have been described elsewhere (Koyro & Stelzer 1988).

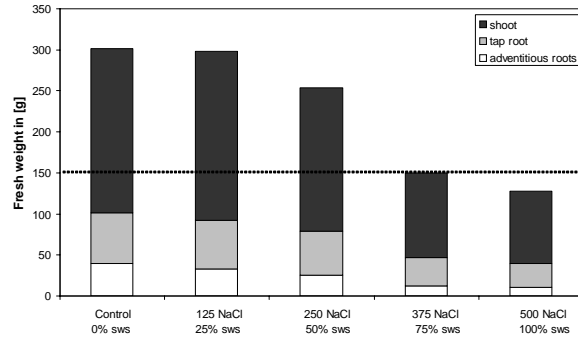
*Statistical treatment*

Data ( $n \geq 6$ ) were subjected to one-way analysis of variance using pc-stat computer software. An analysis of variance was conducted, and least significant differences (LSD) were determined with F test ( $p=0.05$ ).

**Results**

Seabeet growth was reduced through NaCl treatment by more than 50% (Fig. 1). In tap roots the maximal diameter was reduced more than was the length. However, more than any other part of the seabeet, it was especially the storage capacity of the tap root that was reduced. This organ is the most interesting part from an economic point of view, as well as being a depot for the biennial plant. The salinity tolerance was measured on the basis of two growth-related parameters: a) the maximum yield of *Beta vulgaris ssp. maritima* was reduced by 50% ( $C_{50}$ -value) at 375 mol m<sup>-3</sup> NaCl-salinity; b) the salinity threshold was reached at 125 mol m<sup>-3</sup> NaCl-salinity.

Gas exchange parameters such as net

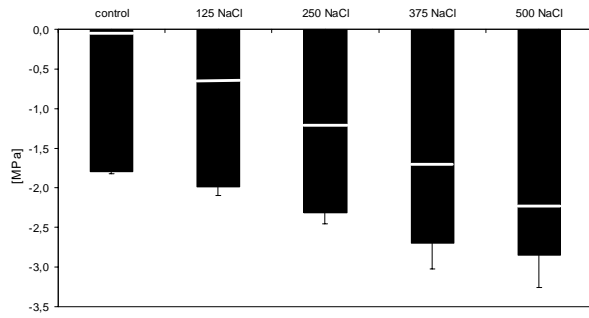


**Fig. 1.** Development of plant freshweight at treatments with different percentages of seawater salinity. The dotted lines reflects the NaCl-salinity where growth depression drops to 50 % of the control plant (threshold of NaCl-salinity according to Kinzel 1982). It is, additionally, a clear proof of the influence of NaCl-salinity that shoot growth was more depressed than root growth (Marschner 1995).

photosynthesis ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ ), stomatal conductance ( $\text{mol m}^{-2} \text{s}^{-1}$ ) and transpiration ( $\text{mol m}^{-2} \text{s}^{-1}$ ) were highest at 250 mol m<sup>-3</sup> NaCl-salinity (Table 1). At salinities above the  $C_{50}$ -value  $C_i/C_a$  ratios, transpiration and stomatal conductance showed a marked reduction and water-use efficiency a steep rise. The controls had a much smaller leaf surface per plant than per single leaf and a higher ratio of leaf mass to area (LAR, defined as weight per cm<sup>2</sup>) than plants growing at 500 mol m<sup>-3</sup> NaCl-salinity (results not shown). These changes support a more

**Table 1.** Gas-exchange parameters such as transpiration rate, stomatal conductance, CO<sub>2</sub>-net assimilation (photosynthesis) rate, water-use efficiency (of photosynthesis) and  $C_i/C_a$ -ratio ( $C_i$ : intracellular CO<sub>2</sub>-concentration;  $C_a$ : atmospheric CO<sub>2</sub>-concentration) in juvenile leaves of *Beta vulgaris ssp. maritima* at different NaCl-salinities.

Culture conditions	Transpiration (mmol*cm <sup>-2</sup> *s <sup>-1</sup> )	Stomatal conductance (mmol* cm <sup>-2</sup> *s <sup>-1</sup> )	CO <sub>2</sub> net assimilation rate (mmol*cm <sup>-2</sup> *s <sup>-1</sup> )	Water use efficiency of photosynthesis (mmol CO <sub>2</sub> * mmol <sup>-1</sup> H <sub>2</sub> O)	$C_i/C_a$
Control (0% sws)	1.82 ± 1.30	0.09 ± 0.07	2.79 ± 0.22	1.59 ± 0.69	0.93
125 NaCl (25% sws)	1.87 ± 0.64	0.09 ± 0.02	3.13 ± 0.20	1.51 ± 0.56	0.88
250 NaCl (50% sws)	2.12 ± 1.66	0.11 ± 0.09	3.49 ± 0.23	1.43 ± 0.96	0.51
375 NaCl (75% sws)	1.77 ± 0.11	0.04 ± 0.01	2.90 ± 0.10	2.49 ± 0.17	0.67
500 NaCl (100% sws)	0.44 ± 0.27	0.02 ± 0.01	1.58 ± 1.01	3.47 ± 2.44	0.72



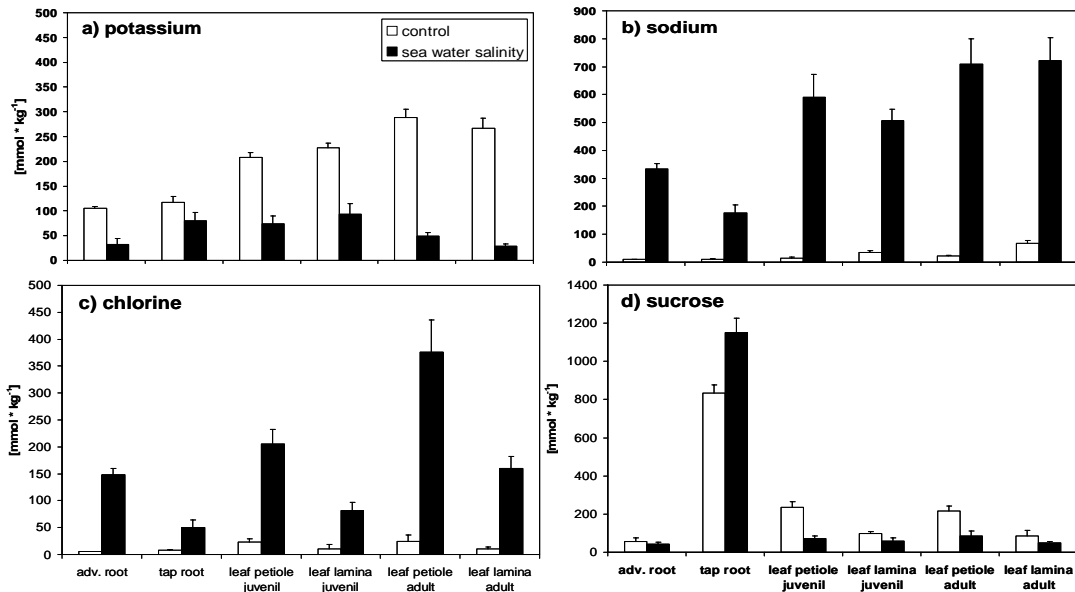
**Fig. 2.** Leaf water potentials (MPa) of *Beta vulgaris* ssp. *maritima*. The white lines in the bars mark the water potentials in the nutrient solutions. Leaf water potentials were always lower than in the assigned nutrient solution potential. The difference between water potentials in the leaves and in the nutrient solutions decreased with increasing NaCl-salinity.

efficient water use (WUE) mainly by reduction of the transpiration area.

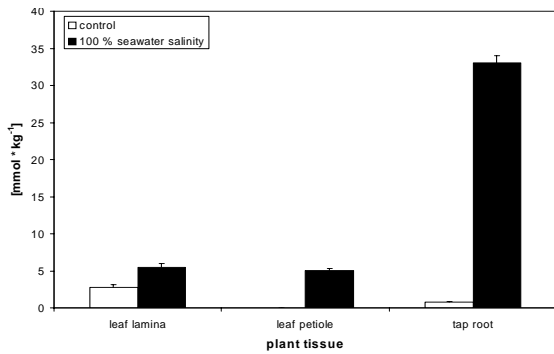
Data on the leaf water potential demonstrated clearly that *Beta vulgaris* ssp. *maritima* has a sufficient adjustment mechanism even when treated with high salinity, suggesting that there was no reason for growth to be reduced by water

deficit (Fig. 2). Thus, if the rate of supply of water to the shoot is not restricted, the depression of shoot growth shown in Fig. 1 is likely to be connected to mineral nutrition. Reduction of the water potential was obtained mainly by increasing the concentration of osmotically active solutes. The decrease in leaf water potential (petiole and lamina) after adding NaCl was compensated for mainly by an uptake of Na and Cl (Fig. 3b and c). At the same time, however, the concentration of K decreased significantly in all tissues of salt-treated plants (Fig. 3a). It seems to be a matter of controversy as to whether a decrease in the amounts of nutrients or unfavorable nutrient ratios (e.g. Na<sup>+</sup>/K<sup>+</sup>) is an important factor in impaired growth (such as leaf elongation).

NaCl salinity led to a reduction in the maximum storage capacity of the tap root, the economically most interesting part of the seaboot. Sucrose was the most abundant carbohydrate in the root (Fig.3d). NaCl-salinity led to a significant increase in sucrose in the tap root and a marked decrease of the same in the leaf regions. Solely the sucrose pool of the tap root was overabundant for an osmotic adjustment of this organ at high NaCl salinities. Additionally, 500 mol m<sup>-3</sup> NaCl-salinity



**Fig. 3.** Potassium- (a), sodium- (b), chlorine- (c) and sucrose concentrations (d) in mmol \* kg<sup>-1</sup> fW (fresh weight) in different tissues of *Beta vulgaris* ssp. *maritima* at different NaCl-salinities. The osmotic adjustment in leaves and adventitious roots is based mainly on the accumulation of Na and Cl. Soluble carbohydrates were mainly responsible for the osmotic adjustment of the taproot. 0% sea water salinity = white bars, 100% sea water salinity = black bars. Adv. Root = adventitious roots, LP = leaf petiole, L = leaf lamina.



**Fig. 4.** Proline concentrations in the juvenile leaf (lamina and petiole) and in the tap root of *Beta vulgaris* ssp. *maritima* at different NaCl-salinities. 0% sea water salinity = white bars, 100% sea water salinity = black bars.

led in the tap root to the lowest Na and Cl and the highest K concentrations of all plant tissues. NaCl salinity produced an increase in proline in leaf and root tissues (Fig. 4). The highest proline concentration was measured in the tap root at 500 mol m<sup>-3</sup> NaCl-salinity (33.03 mmol \* kg FW). Proline could thus be a valuable compatible solute in the cytoplasm of the tap root of salt-treated seabees.

In summary, there was no proof for the existence of nutrient imbalance or ion toxicity (e.g. Na or Cl) in the tap root. The reason for the reduction in tap root growth would appear to lie in the leaves. There was still a high selectivity for the accumulation of K in the leaves of salt-treated plants. However, the question arose of how specific and essential functions of this element can be maintained under these conditions.

The general scientific data provides a general impression of the various mechanisms involved in adaptation to high NaCl-salinity. However, they do not explain the seabee's particular threshold of

salinity tolerance. The analysis of the distribution of elements (compartmentation) can be one way of detecting such limiting factors. EDXA measurements of the vacuolar and cytoplasmic content of individual epidermal cells of bulk frozen control leaves give evidence for several differences between the two compartments (Table 2). The vacuolar K-, Mg-, P and S-concentrations in control treatments are significantly higher in comparison with the cytoplasm. The leaf-vacuoles in their entirety can be described as a capacious gathering pool for K, Mg, P and S, with high storage capacity for sodium and chloride. This pool is needed in cases of high NaCl-salinity in order to maintain mineral homeostasis in the cytoplasm. High NaCl-salinity seems to lead in this compartment to a breakdown of homeostasis because of P, S and K-deficiency and not to a flooding with Na or Cl. Significant concentrations of Na and Cl can be detected at 500 mol \* m<sup>-3</sup> NaCl-salinity only in the vacuole. However, these concentrations are still sufficient for the osmotic adjustment of this compartment.

### Discussion

Salinity strongly influenced gas-exchange properties. The growth and net photosynthesis rates of *Beta vulgaris* ssp. *maritima* varied in the same manner with increasing salinity. At a high salinity level (mol m<sup>-3</sup> NaCl-salinity), growth reduction was 57.6% (Fig. 1), while the reduction in net photosynthesis was only 54.7% (Table 1). Several authors have indicated that several parameters may be involved in growth reduction at high NaCl salinity (Flowers 1985). Since growth declined in the same range as net photosynthesis, it is the decline in photosynthesis that explains growth inhibition at high salinity levels. However,

**Table 2.** Sodium- (Na) magnesium- (Mg), phosphorus- (P), sulphur- (S), chlorine- (Cl) and potassium- (K) concentrations in mol m<sup>-3</sup> (measured with EDX-analysis in bulk frozen tissues) in the vacuoles and in the cytoplasm of adaxial epidermis cells of *Beta vulgaris* ssp. *maritima*.

Cell compartment	Treatment	Na	Mg	P	S	Cl	K
Vacuole	Control	12.24	23.96	22.19	40.42	22.60	282.45
		±3.11	±1.22	±4.57	±7.90	±4.65	±14.70
	500 NaCl	724.85	4.34	6.40	2.74	654.29	9.29
		±65.08	±1.66	±4.15	±1.88	±54.79	±6.12
Cytoplasm	Control	bdl	bdl	58.19	10.53	bdl	88.94
				±8.43	±3.99		±9.50
	500 NaCl	bdl	bdl	28.11	bdl	bdl	66.71
				±6.71			±7.22

one cannot separate the effects of salinity on growth and on photosynthesis.

Gas exchange parameters such as net photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $\text{mol m}^{-2} \text{s}^{-1}$ ), and transpiration ( $\text{mol m}^{-2} \text{s}^{-1}$ ) were highest at  $250 \text{ mol m}^{-3}$  NaCl-salinity. At salinities above the  $C_{50}$ -value  $C_i/C_a$  ratios, transpiration and stomatal conductance showed a distinct reduction and water-use efficiency a marked increase. Decreased stomatal conductance diminished transpiration rates, thereby improving water-use efficiency. This tended to reduce the salt loading into the leaves and helped increase longevity by maintaining salts at subtoxic levels longer than would be the case if transpiration rates were not diminished (Everard *et al.* 1994). At  $375 \text{ mol m}^{-3}$  NaCl-salinity stomatal conductance was severely reduced, and impairment of carboxylation capacity was evident from net photosynthesis rate and  $C_i/C_a$  ratio. Since numerous factors may have contributed to the decline in net photosynthesis during treatments with higher salinity, it is difficult to determine whether stomatal or nonstomatal factors were dominant. Na cannot wholly replace K for stomatal opening, since a sodium channel in plants was not identified and stomatal opening requires channels. However, stomatal factors were apparently effective in limiting net photosynthesis at intermediate salinities, and nonstomatal limitations prevailed under more severe salinity stress (Everard *et al.* 1994). At high salinity levels, a major component was often a reduction in the carbon reduction cycle (Bethke & Drew 1992).

In salt-treated seabiet, the decrease in net photosynthesis may also indicate a reduced capacity to utilize products of light reaction for carbon metabolism. Tissue content is, therefore, linked to photosynthetic carbon anabolism, catabolism and translocation. Stress-induced accumulation may be a symptom of stress rather than an adaptation to it (Fig. 3d).

As shown above, leaf cells of the seabiet exhibit complete regulation of turgor when osmotically stressed with  $400 \text{ mol m}^{-3}$  NaCl. The seabiet was able to balance out low external water potential (Fig. 2) and generated turgor by accumulating high Na and Cl concentrations in the leaf (Fig. 3b and 3c). However, the specificity for the uptake of K (but also of Mg and Ca; for results, see Koyro 2000) was not sufficient to prevent

dilution of this ion at tissue level (Fig. 3a). Our results suggest that the salt-induced changes in ion relations can be tolerated because Na and Cl concentrations are relatively low in the cytoplasm as compared to the vacuole.

Concrete proof for this suggestion is obtainable only from measurements (such as EDXA) at single cell level in the leaf. The vacuolar K concentrations can vary in leaf cells between  $10$  and  $250 \text{ mol m}^{-3}$  (Hsiao & Läuchli 1986). In its osmotic functions in the vacuoles, K is also replaceable by other solutes (such as NaCl in the leaf and sucrose in the taproot), as shown in the present paper. It was shown earlier that the seabiet performs (at low NaCl-salinity) a strictly coordinated storage of the elements K, Mg, P and Ca in the vacuoles of distinct leaf cell types (Koyro & Huchzermeyer 2004). The leaf-vacuoles of all cell types have a high storage capacity for potassium (control). P and Mg (in the high salinity variation, at least P) are accumulated mainly in the vacuoles of photosynthetically active cells. The appearance of large Ca-oxalate crystals in the vacuoles of the seabiet has been interpreted as an internal buffer for Ca (Koyro 2000). These internal K, Mg, P and Ca-pools can be considered as depots to cover essential nutrient requirement under high-salinity conditions in the cytoplasm of leaf cells.

The concentrations of the essential elements Mg and Ca are in the cytoplasm, and are below the detection limit of the EDXA (Table 2). It is thus impossible to draw conclusions about the deficiency of these elements in this compartment at high NaCl-salinity. This is not the case for K and P.  $500 \text{ mol m}^{-3}$  NaCl-salinity led to a significant decrease of K- and P-concentrations in this compartment and to a breakdown of homeostasis. However, there was no significant increase in Na and Cl concentrations. The results shown in this paper support the hypothesis that the major reason for the threshold of salinity tolerance in the seabiet is not ion-toxicity (e.g. of Na or Cl) but ion-deficiency (e.g. of K and P) in the cytoplasm. The K concentrations are in the epidermal cytoplasm of control leaves in an ideal range for enzymatic reactions (Koyro & Stelzer 1988). In almost all cases, cytosolic K concentrations are maintained in the range of  $90$ – $200 \text{ mol m}^{-3}$  (Leigh & Wyn Jones 1984). Cytosolic K-concentrations in the leaf epidermis during high-salinity treatment far exceeded this range,



but it may well be the case that other compatible solutes such as betaine or glycine betaine, (compatible solute of many Chenopodiaceae) could fill this gap (Hanson *et al.* 1985; Marschner 1995). However, in its specific functions in these compartments, K (and P) is not replaceable by other inorganic cations such as Na (Cl) or compatible solutes such as proline (Fig.4). Proline can slow down or prevent dehydration of the cytoplasm but cannot replace K, for example, in its catalytic functions. This result demonstrates the necessity of single-cell measurements for the study of characteristic salinity tolerance mechanisms and threshold levels of individual species.

In higher plants, optimal levels of K and P concentrations are essential, e.g. for certain regulatory enzymes involved in protein synthesis or photosynthesis at various levels (Tester & Blatt 1989). The distinct reduction in net photosynthesis could be connected with the low K and P concentrations in the cytoplasm. Further investigations are necessary to verify this hypothesis.

As shown in this paper, the mechanisms described for enhancing NaCl tolerance in leaves are effective only for a distinct phase but may help the sea-beet to complete its life cycle. The biennial plant *Beta vulgaris* ssp. *maritima* sheds its leaves, and consequently the largest portion of its NaCl-load, after the initial vegetation period. The sea-beet builds up a tap root, that part of a biennial plant or herbaceous perennial that allows it to survive the winter (and high NaCl-salinity). In general, parenchyma cells of different organs above or below ground serve as depots for small molecules. The majority of small molecules consist of inorganic ions, organic acids, sugars, a number of nitrogen-containing compounds (like amino acids or alkaloids) and several others. Small molecules are generally stored within the vacuole as solutes. The tap roots are often modified for food storage and are common in biennial plants such as the carrot *Daucus carota* and *Beta vulgaris* ssp. *vulgaris*. The sea-beet is a halophytic ancestor of the sugarbeet (Letschert 1993) and it is thus no surprise that it should store high concentrations of sucrose in its tap root. However, there are two interesting effects of salinity on this depot for small molecules:

1. Salinity leads to an increase in concentrations of energy-conserving substances such as

sucrose and proline. In terms of water conditions, this can be interpreted as an osmotic adjustment in the vacuoles (sucrose) and cytoplasm (proline).

2. Even at high salinity, concentrations of essential minerals such as K, Mg and Ca were much higher and NaCl-concentrations much lower than in other plant organs.

Unlike the sugarbeet, the sea-beet survives irrigation with NaCl-salinities in the range of seawater (Glenn *et al.*, 1999). Under such circumstances, its tap root provides excellent conditions for the second vegetation period (without a high NaCl burden) and a reasonably high potential for sustainable economic halophyte utilisation.

The physiological investigations shown above promise to provide a highly valuable means of detecting mechanisms inhibiting NaCl stress, and may also provide opportunities for screening different varieties for their adaptation to salinity. This statement includes also the variation of ionic conditions in the substrate, duration of exposure, stage of plant development, and plant organ and environmental conditions. This study is a practical first step towards selecting economically important cash-crop halophytes. One possible consequence of the results presented in this paper is the supply of sufficient fertilizers at high NaCl-salinity in order to reduce the symptoms of K-, S- and P-deficiency.

For future studies on the utility of the sea-beet, precise data about the ecological demands of this halophytic species are required. The physiological studies presented at this paper have the potential to provide highly useful means of detecting individual mechanisms employed by the sea-beet to combat NaCl stress, and may also provide opportunities for the comparison and screening of different varieties for their adaptation to salinity. However, this can be only a first step in developing an economically viable plant for saline habitats. The gradual implementation of the following areas could be one way to establish this potentially useful cash crop halophyte:

- (a) Greenhouse experiments on the local substrates (and under local climatic conditions) to select and propagate promising sites.
- (b) Studies with lysimeters on field site to observe water consumption and ion movement.

- (c) Design of a sustainable production system on plantations in coastal areas or at inland sites (for example, for economic purposes).
- (d) Testing yield and (market) acceptance of the product.

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