

Physiology / Physiologie

## Comparative effect of potassium on K and Na uptake and transport in two accessions of *Arabidopsis thaliana* during salinity stress

Rym Kaddour<sup>a,\*</sup>, Nawel Nasri<sup>a</sup>, Sabah M'rah<sup>a</sup>, Pierre Berthomieu<sup>b</sup>, Mokhtar Lachaâl<sup>a</sup>

<sup>a</sup> *Physiologie et biochimie de la tolérance au sel des plantes, faculté des sciences de Tunis, campus universitaire, 1060 Tunis, Tunisia*

<sup>b</sup> *UMR biochimie & physiologie moléculaire des plantes, Montpellier SupAgro, Institut national de la recherche agronomique, Centre national de la recherche scientifique, Université Montpellier II, place Viala, 34060 Montpellier cedex 1, France*

Received 1 March 2009; accepted after revision 8 May 2009

Available online 8 August 2009

Presented by Philippe Morat

### Abstract

Potassium-sodium interaction was compared in two natural accessions of *Arabidopsis thaliana*, Columbia-0 and NOK2. Seedlings were grown in the presence of 0 or 50 mM NaCl and 0.1; 0.625 or 2.5 mM K<sup>+</sup>. At the lowest K<sup>+</sup> concentration, salt treatment inhibited both K<sup>+</sup> uptake and growth. Increasing the K<sup>+</sup> availability did not modified salt response in Columbia-0, but restored nearly normal net K<sup>+</sup> uptake in NaCl condition and alleviated NaCl growth reduction in NOK2. The effect of K<sup>+</sup> and NaCl on transcript level of several K<sup>+</sup> and Na<sup>+</sup> transporters in both shoots and roots was assessed using semi-quantitative RT-PCR. The mRNA abundance of the *NHX1* and *SOS1* Na<sup>+</sup>/H<sup>+</sup> antiporters was significantly increased by 50 mM NaCl in the two accessions. *NHX1*, which is responsible for Na<sup>+</sup> sequestration into vacuoles, was more up-regulated in NOK2 leaves than in Columbia-0's in NaCl stress condition. *AKT1*, which is the major channel involved in K<sup>+</sup> absorption, was down-regulated in salt stress condition, but was not responding to K<sup>+</sup> treatments. Only in NOK2, *SKOR* and *AKT2*, which respectively control xylem and phloem K<sup>+</sup> transport, were markedly up-regulated by 2.5 mM K<sup>+</sup> in both roots and shoots, independently of NaCl. Phenotypic and gene expression analyses suggest that the relative salt tolerance of NOK2 is mainly due to a high ability to sequester Na<sup>+</sup> in the vacuole and to take up and transport K<sup>+</sup>. Up-regulation of *SKOR* and *AKT2* by K<sup>+</sup>, and of *NHX1* by NaCl could participate in determining this phenotype. **To cite this article:** R. Kaddour et al., C. R. Biologies 332 (2009).

© 2009 Published by Elsevier Masson SAS on behalf of Académie des sciences.

### Résumé

**Comparaison de l'interaction K/Na sur leur apport et transport entre deux accessions d'*Arabidopsis thaliana* lors d'un stress salin.** L'interaction potassium-sodium est comparée entre deux lots d'*Arabidopsis thaliana*, référencés Columbia-0 et NOK2, issus de population naturelle. Les plantes sont cultivées en présence de 0.1 ; 0.625 ou 2.5 mM K<sup>+</sup>, avec ou sans NaCl 50 mM. A la plus faible concentration de K<sup>+</sup>, le sel diminue l'absorption de K<sup>+</sup> et la vitesse de croissance. L'augmentation de la concentration de K<sup>+</sup> en présence de sel ne modifie pas l'absorption de cet ion chez Columbia-0, mais elle la rétablit à sa valeur normale, tout en restaurant la croissance chez NOK2. Les effets de K<sup>+</sup> et de NaCl sur l'abondance des transcrits de quelques systèmes de transport de K<sup>+</sup> et Na<sup>+</sup> dans les parties aériennes et les racines sont étudiés à l'aide d'une RT-PCR semi-quantitative. Le niveau de transcription des gènes des deux antiports Na<sup>+</sup>/H<sup>+</sup> *NHX1* et *SOS1* est significativement augmenté en présence de NaCl pour les deux accessions. *NHX1*, antiport responsable de la séquestration de Na<sup>+</sup> dans les vacuoles, est davantage exprimé dans les feuilles

\* Corresponding author.

E-mail address: [rym\\_kaddour@yahoo.fr](mailto:rym_kaddour@yahoo.fr) (R. Kaddour).

de NOK2 par comparaison avec Columbia-0, en présence de NaCl. Quant à AKT1, canal majeur impliqué dans l'absorption de  $K^+$ , il est sous exprimé en présence de sel, mais il est insensible à la variation de la concentration de  $K^+$ . Chez NOK2, *SKOR* et *AKT2*, qui codent des canaux impliqués dans le contrôle du transport de  $K^+$  respectivement dans le xylème et le phloème, sont surexprimés en présence de 2.5 mM  $K^+$  dans les racines et les feuilles, indépendamment de NaCl. Cette comparaison des phénotypes et de l'expression des gènes suggère que la tolérance au sel de *NOK2* est due essentiellement à sa forte capacité de séquestrer  $Na^+$  dans les vacuoles et de prélever et de transporter  $K^+$ . La surexpression de *SKOR* et *AKT2* par  $K^+$ , et de *NHX1* par NaCl pourraient participer dans la détermination de ce phénotype. **Pour citer cet article : R. Kaddour et al., C. R. Biologies 332 (2009).**

© 2009 Published by Elsevier Masson SAS on behalf of Académie des sciences.

**Keywords:** *Arabidopsis thaliana*; Sodium chloride;  $K^+$  supply; Semi quantitative RT-PCR; Potassium transporters

## 1. Introduction

Potassium is the most abundant inorganic cation in the cytoplasm of all organisms. In plants, it plays a crucial role in osmoregulation, turgor maintenance and cell expansion [1]. Sodium ion can compete with  $K^+$  and inhibit transport and metabolic processes depending on  $K^+$ . Since  $Na^+$  is frequently present at higher concentrations than  $K^+$  in soils, most plants must have transport systems with high selectivity for  $K^+$  against  $Na^+$  [2]. Plants also possess transport systems that compartmentalize  $Na^+$  into the vacuole. This is an essential function in protecting the cytosolic biochemical machinery and ensuring a balance against the low extracellular osmotic potential created by salt stress [3,4]. Plants feature both inter-specific and intra-specific variations for  $K^+$  and  $Na^+$  transport. NaCl-tolerant varieties generally maintain a high cytosolic  $K^+/Na^+$  ratio, when they are in presence of salty medium [5–9]. Considering the  $K^+$  vs.  $Na^+$  selectivity as the ratio between the  $K^+/Na^+$  ratio in leaves and the  $K^+/Na^+$  ratio in the soil solution, a diversity analysis performed in *A. thaliana* evidenced a strong correlation between salt tolerance and  $K^+$  vs.  $Na^+$  selectivity [10]. The two accessions displaying extreme phenotypes were Columbia-0, which showed a 50% reduction in shoot dry weight and a  $K^+$  vs.  $Na^+$  selectivity of 1 in response to salt stress, and NOK2, which showed no reduction in shoot dry weight and a  $K^+$  vs.  $Na^+$  selectivity of 5.

The causes of the  $K^+$  vs.  $Na^+$  selectivity remain unclear. At least 35 genes present in the *Arabidopsis* genome are thought to encode various  $K^+$  channels or transporters [11]. The actual functions of some of these genes have been inferred from reverse genetic approaches, in which gene disruption was associated with lower  $K^+$  content and reduced cell or whole plant growth as compared to wild type [12–15]. Passive uptake of  $K^+$  into roots is mediated by  $K^+$ -selective channels located on the plasma membrane of root cells such

as AKT1 [12,14,16,17]. *AKT1* disruption inhibits *Arabidopsis* growth at low  $K^+$  concentration [13]. This gene is differently regulated by salt in salt-tolerant and salt-sensitive rice varieties [8]. Potassium uptake is also ensured by the *HAK5* gene. This transporter contributes to high-affinity potassium uptake in *Arabidopsis* roots [17]. Long distance transport of  $K^+$  involves *SKOR* [18] and *AKT2* [19], two channels involved in  $K^+$  secretion into the xylem in roots and into the phloem in shoots, respectively. Both *AKT1* and *SKOR* are present at loci that coincide with QTLs for  $K^+$  content in *Arabidopsis* leaves [20].

Information relative to the transporters involved in sodium transport is more limited. Vacuolar compartmentalization of  $Na^+$  is achieved by the action of  $Na^+/H^+$  antiporters present on the tonoplast. *AtNHX1* is one of these antiporters; it can mediate  $Na^+$  and  $K^+$  coupled transport in vacuoles [21] and its over-expression improves plant salt tolerance [21–23]. *AtHKT1* has been shown to control  $Na^+$  homeostasis and to regulate potassium nutrient status in planta [24]. Recently, another  $Na^+/H^+$  antiporter (*NHX5*) has been identified as playing a role in salt tolerance by controlling the accumulation of  $Na^+$  and  $K^+$  in shoots [25]. Sodium can also be moved out of the cell by *SOS1*, which encodes a plasma membrane  $Na^+/H^+$  antiporter controlling long distance  $Na^+$  translocation from root to shoot in *Arabidopsis*. Over-expressing *SOS1* in this species increases salt tolerance [26].

The purpose of this study was to explore the combined effects of a mild salt stress and increasing  $K^+$  supplies on the two *Arabidopsis thaliana* natural accessions, Columbia-0 and NOK2, at the phenotypic and molecular levels. A semi-quantitative RT-PCR method was used to monitor changes in the transcript abundance of the *AKT1*, *AKT2*, *SKOR*, *NHX1* and *SOS1*  $K^+$  and  $Na^+$  transporters in relation to the varying  $Na^+$  and  $K^+$  treatments.

## 2. Materials and methods

Seeds of NOK2 and Columbia-0 accessions provided by the *Nottingham Arabidopsis Stock Centre* (references N1403 and N907, respectively), were grown in pots containing a 1:2 (v:v) mixture of sand and peat, and placed in a culture chamber with a 12 h photoperiod ( $150 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR). The substrate contained 4.9 mg/Kg of  $\text{K}^+$  and 4.6 mg/Kg  $\text{Na}^+$ . The seedlings were irrigated with distilled water during the first 10 days, and then with a nutrient solution [27] diluted to the quarter and composed of: 1.0 mM Mg  $\text{SO}_4$ , 0.625 mM,  $\text{KH}_2\text{PO}_4$ , 0.5 mM  $\text{Ca}(\text{NO}_3)_2$ ; 1.25 mM  $\text{NH}_4\text{NO}_3$ ; 0.04  $\mu\text{M}$   $\text{MnSO}_4$ , 0.5  $\mu\text{M}$   $\text{ZnSO}_4$ , 0.05  $\text{H}_3\text{BO}_3$ , 0.02  $\text{MoO}_3$  at 3  $\mu\text{M}$  FeEDTA, during 13 days. The first harvest was then performed. The plants were thereafter irrigated with the same nutrient solution modified to contain 0.1, 0.625 or 2.5 mM final potassium concentration and 0 or 50 mM NaCl. In the control medium, potassium was added as 0.625 mM  $\text{KH}_2\text{PO}_4$ . In the treatment with the lowest  $\text{K}^+$  concentration, potassium was added as 0.1 mM  $\text{KH}_2\text{PO}_4$ , and the medium was supplemented with 0.525 Na  $\text{H}_2\text{PO}_4$  to maintain P level. Seventeen days later, plants were harvested. For RT-PCR analyses, samples were frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$ . Leaves and roots were analyzed separately.

### 2.1. Phenotypic and elemental analyses

Fresh and dry matter of leaves and roots were determined. For each rosette, all the leaves were cut and photographed, and the individual leaf surface areas were measured using the Optimas<sup>®</sup> software. Roots were collected by submerging the whole root system with the substrate attached to it in 120 ml distilled water. Three rinsing steps were sufficient to get rid of the substrate and rinse the roots. Roots and leaves of each plant were then separately digested in 0.1N  $\text{HNO}_3$ . The concentrations of  $\text{K}^+$  and  $\text{Na}^+$  in the clear extracts were measured by flame photometry (Jenway PFP7, using butane air flame).

According to [28], rate of ion net uptake ( $J_{\text{upt}}$ ) was calculated for each time interval, as

$$J_{\text{upt}} = \Delta I_{\text{tot}} \text{Ln}(Wr_2/Wr_1) / \Delta Wr \Delta t$$

where  $\Delta$  stands for the difference between times  $t_2$  and  $t_1$  (days),  $I_{\text{tot}}$  is the average amount of ion per plant (mmol) calculated from ion concentrations ( $\text{mmol g}^{-1}$  DW) and plant dry weights ( $Wr$ , g).  $\text{Ln}(Wr_2/Wr_1)/Wr$  is the logarithmic mean of the root dry weight (g) calculated over the  $\Delta t$  interval. Logarithmic mean was used

instead of arithmetic mean, because growth kinetics was expected to be exponential rather than linear within the studied time interval.

### 2.2. Statistics

Data are presented as the mean of nine plants for each treatment. Significant differences between treatments were analysed using ANOVA and mean comparison with Duncan post hoc test (Statistica<sup>®</sup>). Values were calculated at the  $P < 0.05$  probability level.

### 2.3. RNA isolation

Plant material (3 plants pooled) was ground in liquid nitrogen, then resuspended in a 0.1 M Tris-HCl, pH 9 buffer containing 1% (w/v) SDS, 10 mM EDTA and 50 mM  $\beta$ -Mercaptoethanol. The mixture was extracted twice with phenol: chloroform:isoamyl alcohol (25:24:1, v:v:v). One tenth volume of 3M sodium acetate pH 5.2 and 2.5 volumes of ethanol were added to the supernatant and the mixture was left on ice for at least one hour for nucleic acid precipitation. After centrifugation at 15,000 g for 20 min, the pellet was resuspended in deionised water. For RNA precipitation, one volume of 4 mM LiCl was added and the mixture was placed on ice for a minimum of two hours. After 45 min of 15,000 g centrifugation, RNA pellets were air-dried for 15 to 30 min, and resuspended in diethyl pyrocarbonate-treated water. RNA was quantified by spectrophotometry. To ensure the quality of RNA, samples were assessed by formaldehyde-denaturing agarose gel electrophoresis.

### 2.4. Semi-quantitative RT-PCR

For reverse transcription, 1  $\mu\text{g}$  RNA was incubated with 0.5  $\mu\text{g}$  oligo-dT at  $70^\circ\text{C}$  for 10 min. The reaction mixture was then brought to 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM  $\text{MgCl}_2$ , 10 mM DTT, 0.5 mM each dNTP and 200 U M-MLV reverse transcriptase (Promega) and incubated at  $42^\circ\text{C}$  for 1 hour. The reaction was stopped by a 15 min long incubation at  $70^\circ\text{C}$ . PCR reactions were conducted using an Eppendorf thermal cycler at  $94^\circ\text{C}$  for 2 min to denature the samples, followed by 30 PCR cycles (30 s at  $94^\circ\text{C}$ , 45 s at  $60^\circ\text{C}$ , 1 min at  $72^\circ\text{C}$ ), and a 5 min final extension step at  $72^\circ\text{C}$ . The number of amplification cycles (30) was determined to ensure that all amplification reactions were stopped in the exponential phase. The primers cited in Table 1 were designed for the gene-specific amplification of transcripts. They were designed to span

Table 1

Forward and reverse specific primers used for RT PCR amplification and size of the amplified fragment.

Gene	Primer sequence (5' → 3')		Product length (pb)	
			Genomic DNA	cDNA
NHX1 At5g27150	Forward	ACCGGTCTGATAAGTGCCTATGTTATC	1464	911
	Reverse	GTTACCCTCAAGCCTTACTAAGATC		
SOS1 At2g01980	Forward	CAAATAAAATGACGACTGTAATCGAC	1551	966
	Reverse	GTCCTTGCAAATGCAGCATAAAAC		
AKT1 At2g26650	Forward	CCGCAACTTCAACTACTTTTGGGTCCGATGCG	591	510
	Reverse	GGGAGTGCATCAAGAGTTTCTTGCTGTTGCAG		
AKT2 At4g22200	Forward	CAGGAGAAATATAATGGACCTCAAG	408	294
	Reverse	GGGTAAACCCACGCAGAGTATG		
SKOR At3g02850	Forward	CGTCGAGCTTTATTGCACTCAC	510	431
	Reverse	GCAATCCGCAAATGTCCGGTAATCTGACC		
Ef1 $\alpha$ At1g07920	Forward	ATGGGTTAAAGAGAAGTTTCACATC	830	730
	Reverse	CCAATCTTGTAGACATCCTGAAG		

PCR reactions of each cDNA were carried out as described in material and methods.

an intron to distinguish PCR products generated from cDNA versus genomic DNA. The PCR reaction was resolved by electrophoresis on a 1 to 2% (w/v) agarose gel stained with ethidium bromide. Gel images were photographed and quantified using Image J version 1.37 software for Windows. The relative intensity of each band in each sample was quantified using spot densitometry and the data were calibrated against the quantities of the Ef-1 $\alpha$  gene. mRNA accumulation measurements were done in triplicate for all the genes. mRNA accumulation measurements were done in triplicate for all the genes.

### 3. Results

#### 3.1. Growth responses of the Columbia-0 and NOK2 *A. thaliana* accessions to the combination of variable potassium nutrition and NaCl treatments

The Columbia-0 and NOK2 accessions were not equally sensitive to increases in the K<sup>+</sup> nutrition (Fig. 1). Whether NaCl was present or not in the culture medium, growth of Columbia-0 plants was not significantly altered in response to the increase in K<sup>+</sup> concentration in the medium. In contrast, when the K<sup>+</sup> concentration in the medium was increased from 0.1 mM to 2.5 mM, the final whole plant biomass of the NOK2 accession increased by ~15% in cases where no NaCl was present and by ~30% in the presence of 50 mM NaCl. The growth decrease observed for both accessions in response to salt at 0.1 mM K<sup>+</sup> was suppressed upon increase in the K<sup>+</sup> supply only in NOK2 (Fig. 1). A protective effect against NaCl stress caused by increasing the K<sup>+</sup> supply was thus demonstrated only for the NOK2 accession.

Dissecting the growth response to understand what would be the cause of the observed differences between the two accessions did not give a clear answer. The total leaf surface per plant increased by ~+25% (Fig. 1) in response to the increase in K<sup>+</sup> supply for both accessions. The origin of this increase was different for the two accessions. Columbia-0 produced a greater number of leaves and showed symptoms of necrosis while NOK2 produced larger leaves without necrosis (Fig. 1). These responses were irrespective of the presence or absence of NaCl in the medium. A major difference between the two accessions was the leaf thickness. It was markedly higher for NOK2 than for Columbia-0, and increased by ~25% under NaCl stress in NOK2 while it decreased by ~50% in Columbia-0 (Fig. 2). In addition, increasing the K<sup>+</sup> supply did not significantly change the leaf thickness in NOK2, while it resulted in a decrease in the leaf thickness in Columbia-0. The water content was also significantly differentially altered in an accession-dependent way, mainly in response to the presence of NaCl in the culture medium (Fig. 2). The NOK2 water content was ~1/3 lower than the Columbia-0 one when no NaCl was added to the culture medium. Upon NaCl stress, it reduced by only 20% while the Columbia-0 one reduced by more than 50%, so that the Columbia-0 water content was lower than the NOK2 one upon NaCl stress. In summary, the positive effect on growth of the increase in the K<sup>+</sup> supply could only be observed for the NOK2 accession, more particularly when the plants were grown in presence of NaCl in the culture medium. The origin of this phenotype was probably that increasing the K<sup>+</sup> supply resulted in an increase in the leaf surface area together with the preservation of leaf hydration and leaf thick-

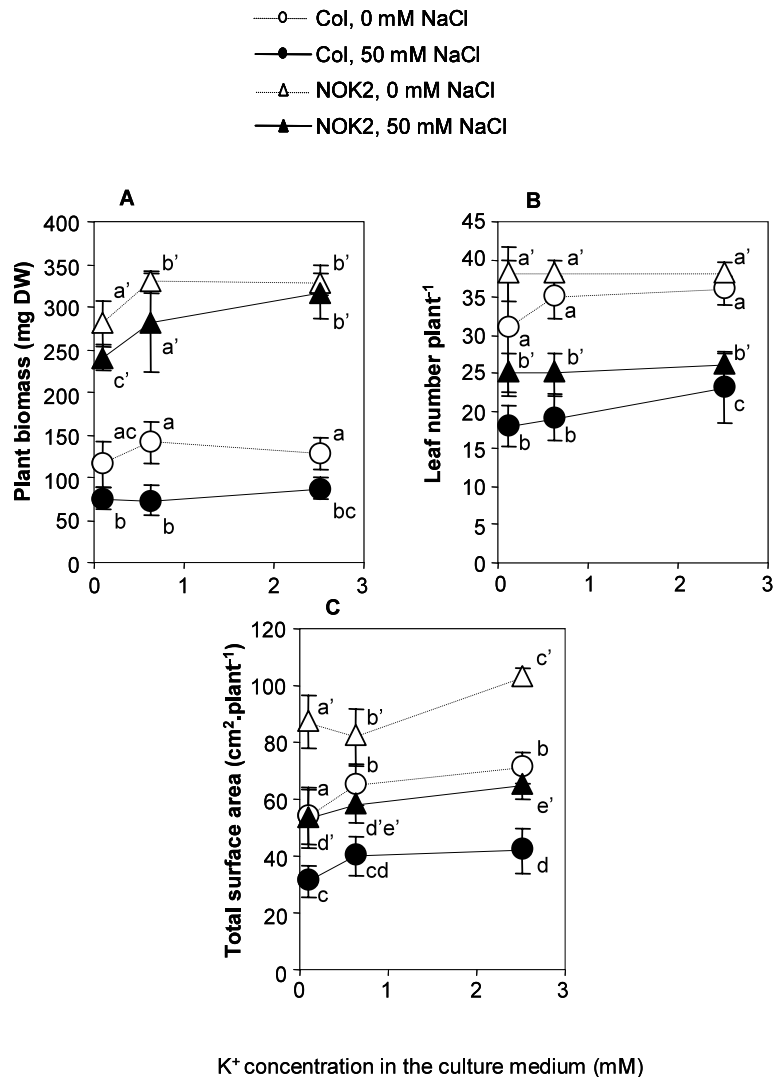


Fig. 1. Combined effect of increasing K<sup>+</sup> concentration in the culture medium and applying or not a 50 mM NaCl treatment on growth of Columbia-0 (Col) and NOK2 plants. The 0.1, 0.625 and 2.5 mM K<sup>+</sup> and 0 or 50 mM NaCl treatments were applied to 23 day-old individual plants and lasted 17 days. 50 mM NaCl: black symbols; 0 mM NaCl: white symbols. NOK2: triangles; Columbia-0: circles. Means ± confidence intervals ( $n = 9$ ;  $p = 0.05$ ).

ness, which positively discriminated *NOK2* in comparison to Columbia-0.

### 3.2. Effect of combining variable potassium nutrition and NaCl treatments on the K<sup>+</sup> and Na<sup>+</sup> net uptake and accumulation in Columbia-0 and NOK2

Leaf K<sup>+</sup> content was strongly dependent on the level of the K<sup>+</sup> supply to the culture medium (Fig. 3), increasing in most cases by +80% to +110% in response to an increase from 0.1 to 2.5 mM in the K<sup>+</sup> concentration in the culture medium. The only exception was for Columbia-0 plants submitted to the -NaCl treatment:

in this case, the leaf K<sup>+</sup> content augmented by only 55% in response to medium enrichment in K<sup>+</sup>. The increase in leaf K<sup>+</sup> content was correlated to a ~25% decrease in the leaf Na<sup>+</sup> content in *NOK2* plants submitted to the NaCl treatment, but it had no impact on the leaf Na<sup>+</sup> content in Columbia-0 plants submitted to the same NaCl treatment (Fig. 3). Notwithstanding the variation in the level of the K<sup>+</sup> supply, *NOK2* plants accumulated ~50% more K<sup>+</sup> and twice less Na<sup>+</sup> in their leaves than Columbia-0 plants, so that the K<sup>+</sup>/Na<sup>+</sup> concentration ratio varied from 0.18 to 0.37 in *NOK2*, and from 0.07 to 0.11 in Columbia-0.

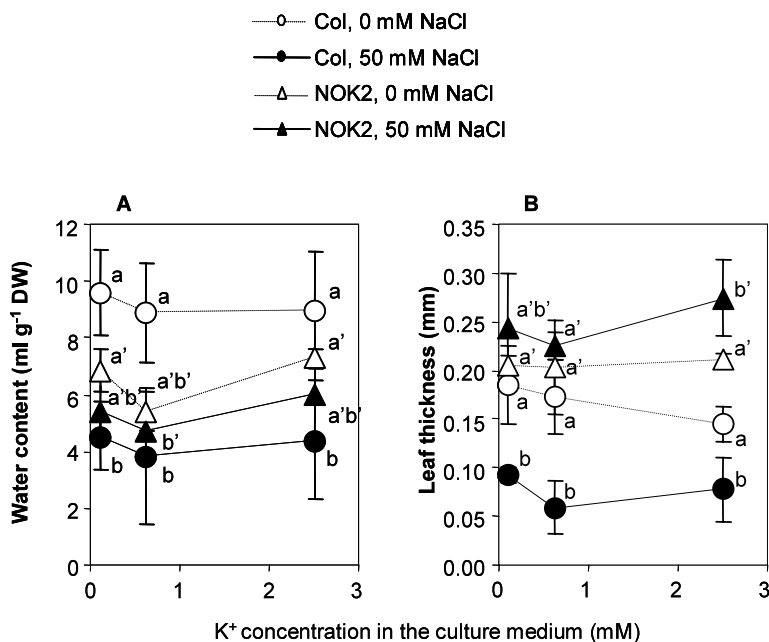


Fig. 2. Combined effect of increasing K<sup>+</sup> concentration in the culture medium and applying or not a 50 mM NaCl treatment on leaf water content and leaf thickness of Columbia-0 (Col) and NOK2 plants. The 0.1, 0.625 and 2.5 mM K<sup>+</sup> and 0 or 50 mM NaCl treatments were applied to 23 day-old individual plants and lasted 17 days. 50 mM NaCl: black symbols; 0 mM NaCl: white symbols. NOK2: triangles; Columbia-0: circles. Means  $\pm$  confidence intervals ( $n = 9$ ;  $p = 0.05$ ).

The rate of ion uptake by roots was estimated by dividing the amount of ion absorbed per plant over the experiment duration to the mean root dry weight. The K<sup>+</sup> net uptake rate was 58% lower, and much more inhibited by NaCl, in Columbia-0 than in NOK2 (Fig. 3). For plants grown on NaCl, the Na<sup>+</sup> net uptake rate was 2 to 30 fold higher than the net K<sup>+</sup> uptake. When K<sup>+</sup> concentration in the culture medium was 0.1 mM, the estimated values of the Na<sup>+</sup> net uptake rate were close to 5 mmol d<sup>-1</sup> g<sup>-1</sup> root DW for both Columbia-0 and NOK2. Increasing the K<sup>+</sup> concentration from 0.1 mM to 2.5 mM resulted in a slight increase of the net Na<sup>+</sup> uptake rate in Columbia-0 and a slight decrease in NOK2 (Fig. 3). In Columbia-0, net K<sup>+</sup> uptake remained equally strongly inhibited by NaCl whatever the external K<sup>+</sup> concentration, while in NOK2, virtually complete alleviation of the NaCl-induced inhibition of net K<sup>+</sup> uptake was obtained by increasing the K<sup>+</sup> concentration in the culture medium (Fig. 3). Altogether these results show that NOK2 was more efficient than Columbia-0 for K<sup>+</sup> uptake, particularly in the presence of NaCl in the culture medium, and that treatment which increased K<sup>+</sup> uptake also slowed Na<sup>+</sup> build-up in NOK2 tissues.

### 3.3. Analyses of the gene expression of selected K<sup>+</sup> and Na<sup>+</sup> transporter

The gene expression of transporters involved in K<sup>+</sup> and Na<sup>+</sup> uptake and in K<sup>+</sup> and Na<sup>+</sup> transport between root and shoot was analysed in Columbia-0 and NOK2 plants grown on media supplemented with the already mentioned different combinations of K<sup>+</sup> and NaCl concentrations. Semi quantitative RT-PCR analyses were done for the *AKT1*, *AKT2* and *SKOR* K<sup>+</sup> channels, and the *NHX1* and *SOS1* Na<sup>+</sup>/H<sup>+</sup> antiporters. Since Ef-1 $\alpha$  had already been demonstrated to show stable expression in plants submitted to varying K<sup>+</sup> or Na<sup>+</sup> external concentrations [17,29], it was used as an internal control. The mRNA abundances of *NHX1* and *SOS1* did not really change in response to the increase in the K<sup>+</sup> concentration in the culture medium. They were however markedly increased in plants submitted to salt stress. *SOS1* mRNA accumulation was 3 times augmented by NaCl, except in Columbia-0 shoots where this augmentation was only 2 to 2.5 times. Salt-induced *NHX1* mRNA accumulation was essentially observed in the shoots. It was two fold higher in NOK2 plants than in Columbia-0 plants. The mRNA abundances of the three K<sup>+</sup> channels were altered differently from each other in response to K<sup>+</sup> supply and NaCl treat-

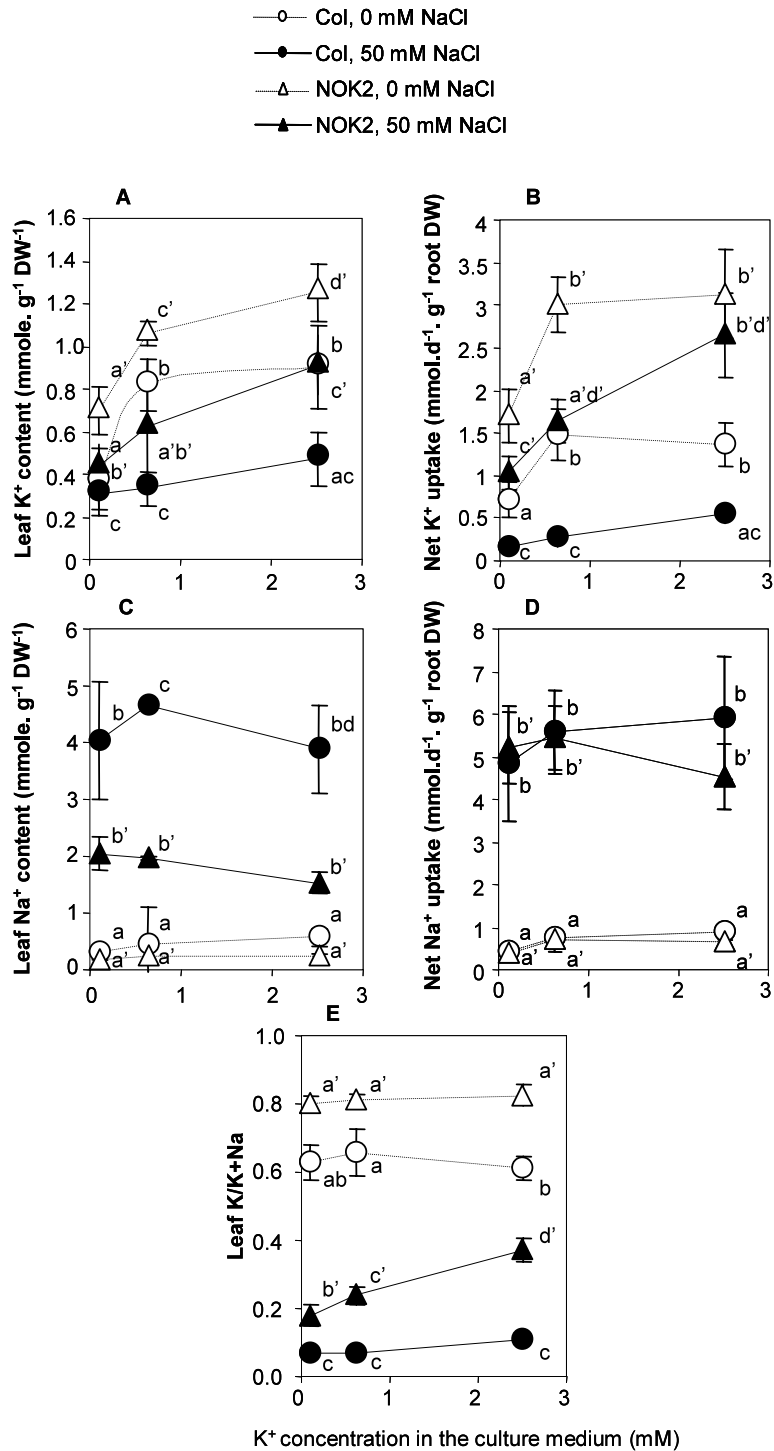


Fig. 3. K<sup>+</sup> and Na<sup>+</sup> accumulation in Columbia-0 (Col) and NOK2 plants. The 0.1, 0.625 and 2.5 mM K<sup>+</sup> and 0 or 50 mM NaCl treatments were applied to 23 day-old individual plants and lasted 17 days. A, C: leaf K<sup>+</sup> and Na<sup>+</sup> contents at the end of the treatments. D, E: net K<sup>+</sup> and Na<sup>+</sup> uptake during the 17-day long treatments. 50 mM NaCl: black symbols; 0 mM NaCl: white symbols. NOK2: triangles; Columbia-0: circles. Means  $\pm$  confidence intervals ( $n = 9$ ;  $p = 0.05$ ). The small amounts of Na<sup>+</sup> absorbed from solutions without added NaCl are exactly accounted for by the Na<sup>+</sup> amounts present in the solid substrate.

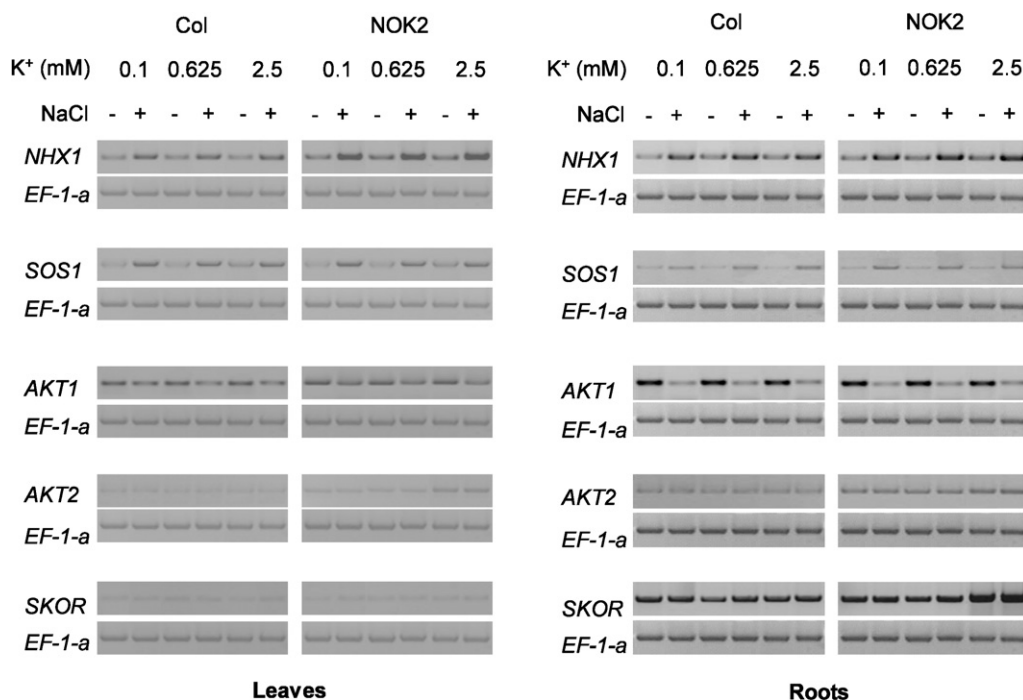


Fig. 4. Semiquantitative RT-PCR analysis of *NHX1*, *SOS1*, *AKT1*, *AKT2* and *SKOR* gene expression in NOK2 and Columbia-0 (Col) plants submitted to combined effects of increasing  $K^+$  concentration in the culture medium and applying or not a 50 mM NaCl treatment. The 0.1, 0.625 and 2.5 mM  $K^+$  and 0 or 50 mM NaCl treatments were applied to 23 day-old individual plants and lasted 17 days. Shoots and roots were analysed separately. Amplification conditions were as described in “Materials and Methods”.

ment. The *AKT1* mRNA abundance in shoot remained the same, whatever the condition considered. *AKT1* expression in roots was however strongly inhibited in response to NaCl stress, whatever the  $K^+$  concentration in the medium. Interestingly, the NaCl-induced reduction in *AKT1* mRNA accumulation was slightly less pronounced in NOK2 roots (~57% reduction) than in Columbia-0 ones (~71% reduction). Almost no change in *AKT2* and *SKOR* mRNA abundance was observed, except in NOK2 in response to 2.5 mM  $K^+$ . Quite surprisingly, in that growing condition, *AKT2* was induced but only in shoots, and *SKOR* was also induced, but only in roots. These responses were irrespective of the presence of NaCl in the culture medium, and were thus specific of the presence of a high  $K^+$  external supply.

In summary, the two  $Na^+/H^+$  antiporters *SOS1* and *NHX1* and the *AKT1*  $K^+$  channel displayed an alteration of their mRNA accumulation only in response to the NaCl treatment, being insensitive to changes in the  $K^+$  supply, while the *AKT2* and *SKOR*  $K^+$  channels showed alteration of their mRNA accumulation only in response to the increasing  $K^+$  supply, being insensitive to the presence or absence of NaCl. There was a marked differential response between the two acces-

sions for all genes: (i) increase in *NHX1* mRNA accumulation in response to NaCl was greater in NOK2 than in Columbia-0; (ii) increase in *SOS1* mRNA accumulation in response to NaCl was less pronounced in Columbia-0 shoots than in NOK2 ones; (iii) decrease in *AKT1* mRNA accumulation in response to NaCl was slightly lower in NOK2 than in Columbia-0 and alteration of *AKT*; (iv) *SKOR* mRNA accumulation was only observed in NOK2.

#### 4. Discussion

The comparison between the salt tolerant NOK2 and the salt sensitive Columbia-0 *A. thaliana* accessions revealed that NOK2 showed a higher salt tolerance than Columbia-0 that could be correlated to a greater  $K^+$  vs.  $Na^+$  selectivity [10]. Here we further analysed these two accessions and compared their performances in response to increasing  $K^+$  concentrations in the culture medium in presence vs. absence of a mild salt stress.

Our analyses confirmed that NOK2 is endowed with greater  $K^+$  vs.  $Na^+$  selectivity than Columbia-0 (Fig. 3E). The  $K^+$  content and the  $K^+$  net uptake rate were both higher in NOK2 than in Columbia-0 plants (Fig. 3), showing that NOK2 has an intrinsic high abil-



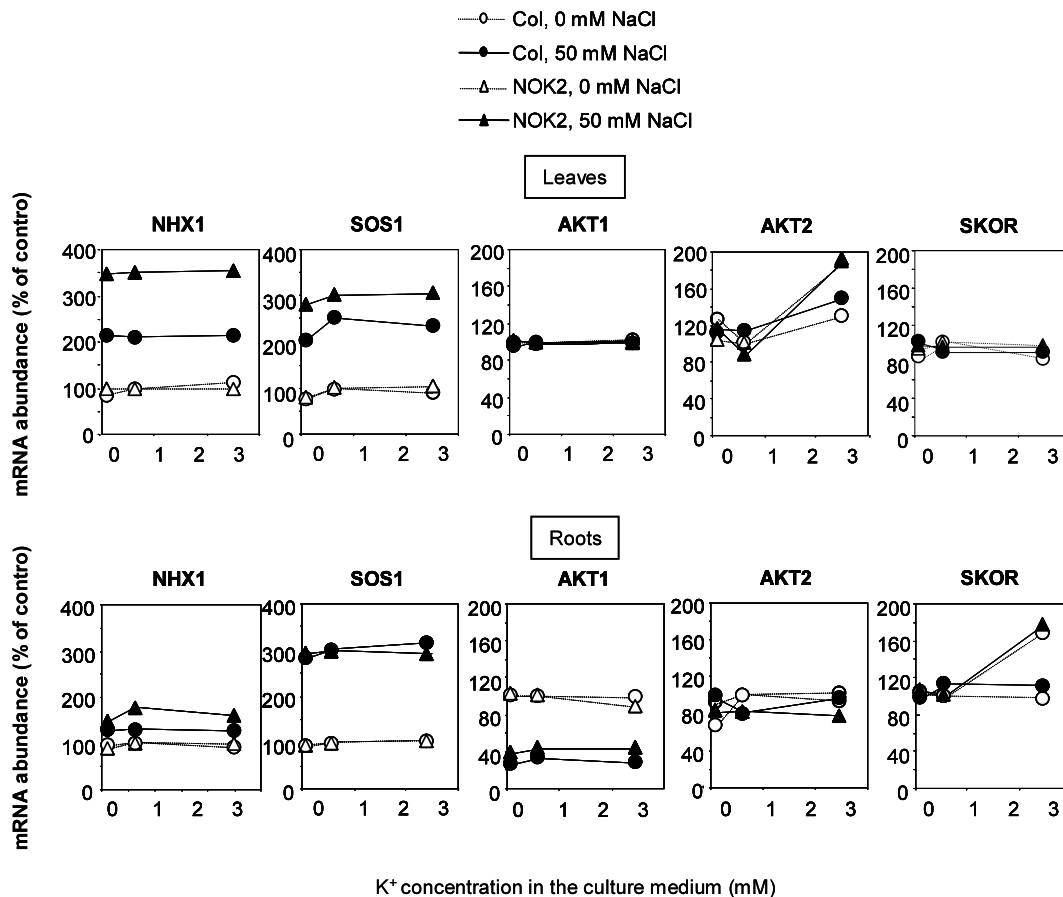


Fig. 5. Combined effect of increasing K<sup>+</sup> concentration in the culture medium and applying or not a 50 mM NaCl treatment on transcript abundances of 5 K<sup>+</sup> and Na<sup>+</sup> transporters in Columbia-0 (Col) and NOK2 plants. The 0.1, 0.625 and 2.5 mM K<sup>+</sup> and 0 or 50 mM NaCl treatments were applied to 23 day-old individual plants and lasted 17 days. Shoots and roots were analysed separately. Raw data from Fig. 5 were quantified using spot densitometry analyses (Image J) and the values were normalized by comparison to the values obtained for Efl- $\alpha$ . Gene expression is given as percentage relative to the value obtained for the same gene in the same organ in response to the treatment corresponding to 0.625 mM K<sup>+</sup> and 0 mM NaCl. Y scales for the 2 Na<sup>+</sup>/H<sup>+</sup> antiporters SOS1 and NHX1 are different from Y scales for the 3 K<sup>+</sup> transporters AKT1, AKT2 and SKOR. 50 mM NaCl: black symbols; 0 mM NaCl: white symbols. NOK2: triangles; Columbia-0: circles. Means  $\pm$  SD ( $n = 3$ ).

ity to absorb K<sup>+</sup> compared to Columbia-0. Increasing K<sup>+</sup> concentrations in the culture medium actually had a positive effect on growth in NOK2 but not in Columbia-0 (Fig. 1A). In response to the NaCl constraint, NOK2 plants showed less reduction in their growth and net K<sup>+</sup> uptake compared to Columbia-0 plants (Figs. 1, 3). Using split root system approaches on glycophyte and halophyte species, several works have demonstrated that nutritional disturbances are implicated in the restriction of the growth under salinity stress [30–32]. Our molecular results showed that the difference in the *AKT1* expression was not so great between the two accessions (Fig. 4), even if *AKT1* is considered as a major contributor to K<sup>+</sup> uptake from the soil solution [12,13,17]. This result is reminiscent of previous reports advancing that *AKT1* is not regulated by changes in the

K<sup>+</sup> availability [12,33]. This suggests that K<sup>+</sup> uptake is controlled at a different level or through the regulation of other(s) transporter(s). In contrast, a NOK2 specific up-regulation was observed for both the *AKT2* and *SKOR* K<sup>+</sup> channel genes at high K<sup>+</sup> concentration (2.5 mM). This was observed for *SKOR* in the roots, and for *AKT2* in the shoot. Indeed, these two K<sup>+</sup> channels are involved in long distance transport of potassium, controlling the K<sup>+</sup> loading into the xylem (*SKOR*) or into the phloem (*AKT2*) [18,19,34]. Over-expression of these two K<sup>+</sup> channels might facilitate the translocation of K<sup>+</sup> from the roots to the transpiring mature leaves (*SKOR*) and then the K<sup>+</sup> circulation from the old leaves to the young ones (*AKT2*). This would contribute to raise the K<sup>+</sup> content to high levels in shoots tissues, which characterizes NOK2 as compared to Columbia-0.

The cause behind the higher salt tolerance of NOK2 in comparison to Columbia-0 was not found to derive from NOK2's greater ability to limit Na<sup>+</sup> absorption by roots, since the net Na<sup>+</sup> uptake was similar in the two accessions. The leaf Na<sup>+</sup> content was however much higher in Columbia-0 than in NOK2 (Fig. 3). This could be the consequence of a lower expression of the *SOS1* Na<sup>+</sup>/H<sup>+</sup> antiporter in Columbia-0 shoots compared to NOK2 ones (Fig. 4). Indeed, *SOS1* controls long distance Na<sup>+</sup> translocation from root to shoot in *Arabidopsis* [26] and over-expression of *SOS1* increases tolerance to NaCl stress [35]. However, *SOS1* over-expression was probably not the main factor responsible for the reduction in leaf Na<sup>+</sup> accumulation observed in NOK2 as compared to Columbia-0. The reduced Na<sup>+</sup> content in NOK2 leaves was more likely a consequence of Na<sup>+</sup> dilution in the larger leaf biomass in this accession. Indeed, NOK2 leaves accumulated more than twice less sodium than Columbia-0 ones and, in the same time, NOK2 plants were two and three times bigger than Columbia-0 ones (Figs. 1, 3). The observed dehydration of Columbia-0 leaves that is concomitant with Na<sup>+</sup> accumulation (Figs. 2, 3) suggests that Na<sup>+</sup> ions transported to these organs were not correctly compartmentalized in leaf cells. In contrast, the observation that Na<sup>+</sup> accumulation was correlated with leaf succulence in NOK2 (Figs. 2, 3) suggested that Na<sup>+</sup> ions imported in leaves participated in retaining water in leaf cells. This could be related to the fact that *NHX1* was over-expressed in response to salt to a higher level in NOK2 than in Columbia-0 (Fig. 4). *NHX1* is indeed responsible for compartmentalizing Na<sup>+</sup> into the vacuole, which participates in maintaining cellular integrity in plants challenged by salt, and attenuates the salt sensitivity in *Arabidopsis* [22,36]. A greater Na<sup>+</sup> storage in the vacuole could enable a greater increase in leaf hydration upon salt treatment in NOK2 plants, while a defect in this function would cause leaf dehydration and necrosis in salt-treated Columbia-0 plants, as it has been proposed for other glycophytes [37–41].

In summary, our results evidence differences in K<sup>+</sup> and Na<sup>+</sup> transports between NOK2 and Columbia-0, which could explain the difference in salt tolerance between the two accessions. NOK2 appears to display a greater cellular and tissular tolerance. This is evidenced by an increase in the water content, leaf thickness and ultimately growth, which discriminates NOK2 from Columbia-0. These phenotypic observations can be correlated with a greater transcript accumulation of the *NHX1* tonoplastic Na<sup>+</sup>/H<sup>+</sup> antiporter gene that would suggest that NOK2 efficiently compartmentalizes Na<sup>+</sup> in its leaf cell vacuoles. However, the main char-

acteristic discriminating NOK2 from Columbia-0 is its superior ability to take up K<sup>+</sup> from the culture medium in the presence of NaCl. The molecular bases for this trait are still unknown, but the two K<sup>+</sup> channels controlling long distance K<sup>+</sup> transport in the plant (*SKOR* and *AKT2*) can be proposed to be candidates for explaining the NOK2 higher performances. Our results enlighten the usefulness of considering NOK2 as a crucial accession for further analysing K<sup>+</sup> nutrition and K<sup>+</sup> vs. Na<sup>+</sup> transport at the molecular level.

## Acknowledgements

This work was supported by a grant from Tunisian–French, CMCU (network 02F/924).

## References

- [1] K. Mengel, E.A. Kirkby, Principles of Plant Nutrition, 5th edition, Kluwer Academic Publisher, ISBN 978-1-4020-0008-9, 1982, p. 849.
- [2] D. Schachtman, W. Liu, Molecular pieces to the puzzle of the interaction between potassium and sodium uptake in plants, Trends Plant Sci. 4 (1999) 281–287.
- [3] M. Tester, R. Davenport, Na<sup>+</sup> tolerance and Na<sup>+</sup> transport in higher plants, Ann. Bot. 91 (2003) 503–527.
- [4] M.P. Apse, E. Blumwald, Na<sup>+</sup> transport in plants, FEBS Lett. 581 (2007) 2247–2254.
- [5] T.J. Flowers, M.A. Hajibagheri, Salinity tolerance in *Hordeum vulgare*: Ion concentrations in root cells of cultivars differing in salt tolerance, Plant Soil 231 (2001) 1–9.
- [6] D.E. Carden, D.J. Walker, T.J. Flowers, A.J. Miller, Single-cell measurements of the contributions of cytosolic Na<sup>+</sup> and K<sup>+</sup> to salt tolerance, Plant Physiol. 131 (2003) 676–683.
- [7] T.A. Cuin, A.J. Miller, S.A. Laurie, R.A. Leigh, Potassium activities in cell compartments of salt-grown barley leaves, J. Exp. Bot. 54 (2003) 657–661.
- [8] D. Gollack, F. Quigley, C.B. Michalowski, U.R. Kamasani, H.J. Bohnert, Salinity stress-tolerant and -sensitive rice (*Oryza sativa* L.) regulate AKT1-type potassium channel transcripts differently, Plant Mol. Biol. 51 (2003) 71–81.
- [9] Y.H. Peng, Y.F. Zhu, Y.Q. Mao, S.W. Wang, W.A. Su, Z.C. Tang, Alkali grass resists salt stress through high [K<sup>+</sup>] and an endodermis barrier to Na<sup>+</sup>, J. Exp. Bot. 55 (2004) 939–949.
- [10] N. Labidi, M. Lachaâl, F. Chibani, C. Grignon, M. Hajji, Variability of the response to NaCl of eight ecotypes of *Arabidopsis thaliana*, J. Plant Nutr. 25 (2002) 2627–2638.
- [11] Z. Qi, E.P. Spalding, Protection of plasma membrane K<sup>+</sup> transport by the salt overly sensitive Na<sup>+</sup>–H<sup>+</sup> antiporter during salinity stress, Plant Physiol. 136 (2004) 2548–2555.
- [12] R.E. Hirsch, B.D. Lewis, E.P. Spalding, M.R. Sussman, A role for the AKT1 potassium channel in plant nutrition, Science 280 (1998) 918–921.
- [13] E.P. Spalding, R.E. Hirsch, D.R. Lewis, Z. Qi, M.R. Sussman, B.D. Lewis, Potassium uptake supporting plant growth in the absence of AKT1 channel activity: Inhibition by ammonium and stimulation by sodium, J. Gen. Physiol. 113 (1999) 909–918.
- [14] M.R. Broadley, A.J. Escobar-Gutierrez, H.C. Bowen, N.J. Willey, P.J. White, Influx and accumulation of Cs<sup>+</sup> by the akt1 mu-

- tant of *Arabidopsis thaliana* (L.) Heynh. lacking a dominant K<sup>+</sup> transport system, *J. Exp. Bot.* 52 (2001) 839–844.
- [15] S. Rigas, G. Debrosses, K. Haralampidis, F. Vicente-Agullo, K. Feldmann, et al., Trh1 encodes a potassium transporter required for tip growth in *Arabidopsis* root hairs, *Plant Cell* 13 (2001) 139–151.
- [16] E.J. Kim, J.M. Kwak, N. Uozumi, J.I. Schroeder, AtKUP1: An *Arabidopsis* gene encoding high-affinity potassium transport activity, *Plant Cell* 10 (1998) 51–62.
- [17] M. Gierth, P. Mäser, J.I. Schroeder, The potassium transporter AtHAK5 functions in K<sup>+</sup> deprivation-induced high-affinity K<sup>+</sup> uptake and AKT1 K<sup>+</sup> channel contribution to K<sup>+</sup> uptake kinetics in *Arabidopsis* roots, *Plant Physiol.* 137 (2005) 105–114.
- [18] F. Gaymard, G. Pilot, B. Lacombe, D. Bouchez, D. Bruneau, J. Boucherez, N. Michaux-Ferriere, J.-B. Thibaud, H. Sentenac, Identification and disruption of a plant shaker-like outward channel involved in K<sup>+</sup> release into the xylem sap, *Cell* 94 (1998) 647–655.
- [19] B. Lacombe, G. Pilot, E. Michard, F. Gaymard, H. Sentenac, J.-B. Thibaud, A Shaker-like K<sup>+</sup> channel with weak rectification is expressed in both source and sink phloem tissues of *Arabidopsis*, *Plant Cell* 12 (2000) 37–51.
- [20] H. Harada, R.A. Leigh, Genetic mapping of natural variation in potassium concentrations in shoots of *Arabidopsis thaliana*, *J. Exp. Bot.* 57 (4) (2006) 953–960.
- [21] H.X. Zhang, E. Blumwald, Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit, *Nat. Biotechnol.* 19 (2001) 765–768.
- [22] M.P. Apse, G.S. Aharon, W.A. Snedden, E. Blumwald, Salt tolerance conferred by overexpression of a vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter in *Arabidopsis*, *Science* 285 (1999) 1256–1258.
- [23] H.X. Zhang, J. Hodson, J.P. Williams, E. Blumwald, Engineering salt tolerant Brassica plants: Characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation, *Proc. Natl. Acad. Sci. USA* 98 (2001) 12832–12836.
- [24] A. Rus, B-ha Lee, A. Muñoz-Mayor, A. Sharkhuu, K. Miura, J.-K. Zhu, R.A. Bressan, P.M. Hasegawa, AtHKT1 facilitates Na<sup>+</sup> homeostasis and K<sup>+</sup> nutrition in planta, *Plant Physiol.* 136 (2004) 1–12.
- [25] L.Y. Shi, H.Q. Li, X.P. Pan, G.J. Wu, M.R. Li, Improvement of *Torenia fournieri* salinity tolerance by expression of *Arabidopsis* AtNHX5, *Funct. Plant Biol.* 35 (2008) 185–192.
- [26] H.Z. Shi, F.J. Quintero, J.M. Pardo, J.K. Zhu, The putative plasma membrane Na<sup>+</sup>/H<sup>+</sup> antiporter SOS1 controls long-distance Na<sup>+</sup> transport in plants, *Plant Cell* 4 (2002) 465–477.
- [27] A.P. Gay, B. Hauck, Acclimation of *Lolium temulentum* to enhanced carbon dioxide concentration, *J. Exp. Bot.* 45 (1994) 1133–1141.
- [28] M.G. Pitman, Whole plants, in: A. Baker, J.L. Hall (Eds.), *Solute Transport in Plant Cells and Tissues*, Longman Scientific and Technical, Harlow, Essex, 1988, pp. 346–385.
- [29] K. Liu, S. Xu, W. Xuan, T. Ling, Z. Cao, B. Huang, Y. Sun, L. Fang, Z. Liu, N. Zhao, W. Shen, Carbon monoxide counteracts the inhibition of seed germination and alleviates oxidative damage caused by salt stress in *Oryza sativa*, *Plant Science* 172 (2007) 544–555.
- [30] D. Messedi, N. Labidi, C. Grignon, C. Abdelly, Limits imposed by salt to the growth of the halophyte *Sesuvium portulacastrum*, *J. Plant Nutr. Soil Sci.* 167 (2004) 720–725.
- [31] H. Attia, N. Karray, R. Mokded, M. Lachaâl, Salt-imposed restrictions on the uptake of macroelements by roots of *Arabidopsis thaliana*, *Acta Physiol. Plant* 30 (2008) 723–727.
- [32] K. Ben Hamed, D. Messedi, A. Ranieri, C. Abdelly, Diversity in the response of two potential halophytes (*Batis maitima* and *Crithmum maritimum*) to salt stress, in: Chedly Abdelly, Muhamed Ashraf, Munir Ozturk, Claude Grignon (Eds.), *Biosaline Agriculture and High Salinity Tolerance*, Birkhäuser Verlag AG, 2008, pp. 71–80.
- [33] F.J.M. Maathuis, V. Filatov, P. Herzyk, C. Krijger, B. Axelsen, S. Chen, B.J. Green, Y. Li, K.L. Madagan, R. Sanchez-Fernandez, et al., Transcriptome analysis of root transporters reveals participation of multiple gene families in the response to cation stress, *Plant J.* 35 (2003) 675–692.
- [34] R. Deeken, C. Sanders, P. Ache, R. Hedrich, Developmental and light-dependent regulation of a phloem-localised K<sup>+</sup> channel of *Arabidopsis thaliana*, *Plant J.* 23 (2000) 285–290.
- [35] H. Shi, B.H. Lee, S.J. Wu, J.K. Zhu, Overexpression of a plasma membrane Na<sup>+</sup>/H<sup>+</sup> antiporter gene improves salt tolerance in *Arabidopsis thaliana*, *Nat. Biotechnol.* 21 (2003) 81–85.
- [36] F.J. Quintero, M.R. Blatt, J.M. Pardo, Functional conservation between yeast and plant endosomal Na<sup>+</sup>/H<sup>+</sup> antiporters, *FEBS Lett.* 471 (2000) 224–228.
- [37] J.J. Oertli, Extra cellular salt accumulation, a possible mechanism of salt injury in plants, *Agrochimica* 12 (1968) 461–469.
- [38] R. Munns, J.B. Passioura, Effect of prolonged exposure to NaCl on the osmotic pressure of leaf xylem sap from intact, transpiring Barley plants, *Aust. J. Plant Physiol.* 11 (6) (1984) 497–507.
- [39] R.W. Kingsbury, E. Epstein, Salt sensitivity in wheat. A case for specific ion toxicity, *Plant Physiol.* 80 (1986) 651–654.
- [40] T.J. Flowers, M.A. Hajibagheri, A.R. Yeo, Ion accumulation in the cell walls of rice plants growing under saline condition: Evidence for the *Oertli hypothesis*, *Plant Cell Environ.* 14 (1991) 319–325.
- [41] M. Speer, W.M. Kaiser, Ion relations of symplastic and apoplastic space in leaves from *Spinacia oleracea* L. and *Pisum sativum* L. under salinity, *Plant Physiol.* 97 (1991) 990–997.