

CONTROL OF K⁺ HOMEOSTASIS: AN ESSENTIAL STRESS TOLERANCE MECHANISM IN PLANTS

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Abstract

Soil salinity is one of the most critical environmental stressors that reduces crop yields worldwide and affects wild plants distribution in nature. Climate change is increasing the salinity of irrigated cropland and natural saline habitats of high ecological value, highlighting the interest in elucidating salt stress tolerance mechanisms in crops and wild plants. One of the well-known adverse effects of salt is the interference of toxic Na⁺ ions with K⁺ uptake and homeostasis, as both cations compete for the same binding sites and transport proteins. Therefore, an increase in substrate salinity is usually accompanied by a reduction of K⁺ concentrations in the plant organs, as it has been observed in many species, both salt sensitive and tolerant. However, in other plants, K⁺ contents are maintained or even increase with increasing Na⁺ concentrations; for example, in some species, K⁺ transport to the leaves is activated at high external salinity to counteract the toxic Na⁺ effects. This review will present several examples of these mechanisms and their relevance for stress tolerance, based primarily on our group's work during the last 20 years.

Key words: climate change, ion toxicity, potassium transport, soil salinity, salt tolerance.

INTRODUCTION

Climate change currently represents the most severe challenge for agricultural production and food security; its effects, such as the increase in average temperatures or the frequency and intensity of extreme weather phenomena - heatwaves, heavy rains and floods, out-of-season frosts or, most important, drought periods - are significantly reducing crop yields worldwide (Liu et al., 2020; Raj et al., 2022; Ray et al., 2019). Along with drought, soil salinity is the most adverse environmental stress factor affecting plant growth and productivity. Climate change also contributes to the progressive salinisation of irrigated cropland in arid and semiarid regions, making good-quality water for irrigation an increasingly scarce resource. Since these regions are the most productive globally - they represent less than 20% of the total farmland but produce more than 40% of our food - the effect of secondary salinisation reducing total food production cannot be ignored (Rabbani et al., 2013; Rahman et al., 2019). The genetic improvement of crop salt tolerance, through

classical breeding and genetic transformation or the newer genome editing techniques, appears to be the most sensible strategy to address this problem (Fita et al., 2015), notwithstanding diverse complementary approaches such as the domestication and commercial cultivation of naturally tolerant wild species (halophytes), 'recovery' of traditional cultivars that could be better adapted to saline soils, or the use of different types of 'biostimulants' that may enhance plant tolerance to abiotic stress (Vicente, 2022). In any case, it is evident that a deep understanding of the mechanisms the plants use as a defence against salt stress will be required, or at least will facilitate enhancing crop salt tolerance and hence food production. Global warming also represents a threat to (wild) plants' biodiversity, as drought and salinity are relevant environmental factors that can substantially modify plant distribution in nature. Coastal and inland salt marshes, littoral dunes, estuaries and other saline environments are highly threatened by human activities - agriculture, industry, urbanisation or tourism - but also by climate change-induced alterations of soil moisture and increased soil salinity.

Many wild species with different degrees of salt tolerance are present in these saline zones, including rare, endemic and endangered taxa of high ecological value. The elucidation of salt tolerance mechanisms in these wild species is also important from a biodiversity perspective, as this knowledge will help design and implement conservation and regeneration programmes for these threatened habitats and maintain the ecosystem services they provide (Dawson et al., 2011; Kidane et al., 2019).

Over the last two decades, our group has been studying the responses to abiotic stress, primarily to drought and salinity, of many different plant species to establish their most effective tolerance mechanisms. We have used both crops and wild plants, combining laboratory, greenhouse and field studies, and ecological, physiological and biochemical approaches. In the following sections, we will briefly describe the most important deleterious effects of salt stress on plants and their general mechanisms of defence against high salinity, which include the control of ion transport and homeostasis. Then, we will focus on those based on the maintenance or even increase of K^+ concentrations in plant organs, in parallel to the increase in Na^+ levels, providing several examples from our own work and the literature.

SALT STRESS EFFECTS ON PLANTS

High salinity causes different deleterious effects on plants associated with its main components: osmotic stress and ion toxicity. The osmotic effect on plants of high ion concentrations in the soil is shared by other abiotic stressors, such as low and high temperatures, heavy metals, or drought. In fact, soil salinity causes a 'physiological drought' in plants, limiting water uptake and inducing dehydration of plant cells and organs (Munns & Tester, 2008; Sheldon et al., 2017).

On the other hand, Na^+ and Cl^- ions are toxic if accumulated at high concentrations in the cytosol as both - although much more attention has been given to the effects of the cation - inhibit many enzyme activities and can also inactivate directly proteins and macromolecular structures, breaking the ionic interactions that maintain their functional conformation; sodium also interferes with essential cellular processes,

such as protein synthesis or mRNA processing (Flowers et al., 2015; Zhang et al., 2013). Another negative effect of salt stress is the interference with mineral nutrition, including the inhibition by Na^+ of K^+ uptake, which will be dealt with more extensively in the following sections of this review.

Finally, as with many other abiotic stressors, high salinity causes oxidative stress as a secondary effect, increasing the cellular levels of reactive oxygen species (ROS). ROS accumulation leads to the oxidation of membrane lipids, amino acid residues in proteins and the DNA bases, causing alteration of membrane permeability, inactivation of proteins, DNA mutations and, eventually, cell death (Apel & Hirt, 2004; Savchenko & Tikhonov, 2021).

PLANT RESPONSES TO SALT STRESS AND SALT STRESS TOLERANCE

All plants, independently of their relative tolerance level, activate a series of basic and conserved physiological, biochemical and molecular mechanisms to respond to salt stress. Those responses attempting to counteract the adverse effects of oxidative stress are triggered by diverse stress conditions and consist of activating different antioxidant systems to reduce toxic ROS levels. They include several enzymes such as superoxide dismutase, catalase, ascorbate peroxidase or glutathione reductase, to mention just some of the most relevant. Salt stress also activates the synthesis of antioxidant metabolites such as vitamin C, carotenoids, glutathione or phenolic compounds, particularly the subgroup of flavonoids (García-Caparrós et al., 2021; Hasanuzzaman et al., 2021).

As mentioned above, osmotic adjustment is also required when the plants are affected by any stress condition that includes an osmotic component. To limit cellular dehydration, the typical plant response is the synthesis and accumulation in the cytosol of compatible solutes or 'osmolytes'. Osmolytes are organic, low-molecular-weight metabolites of diverse chemical nature, which are highly soluble and do not interfere with metabolic reactions even when present at high concentrations (Slama et al., 2015; Szabados & Saviouré, 2010).

Responses specifically directed to reduce the toxic salt effects are based essentially on ion transport and ion homeostasis control: exclusion of ions at the root level, transport to the plant aerial parts, and intracellular distribution, generally involving their removal from the cell cytosol (Bassil & Blumwald, 2014; Zhu, 2003). Ions like Na^+ and Cl^- can contribute to osmotic balance under salt stress, and ion transport requires lower energy consumption than the *de novo* synthesis of organic osmolytes (Raven, 1985); however, they cannot reach high cytosolic concentrations because of their toxicity. In agreement with the "ion compartmentalisation hypothesis" (Flowers et al., 1986), toxic ions should be transported and stored in the vacuoles until a species-specific concentration threshold is reached. This hypothesis is generally accepted, although it has been demonstrated experimentally only in a few cases due to the technical difficulty of measuring ion concentrations in subcellular compartments. Other specific transport proteins are responsible for expelling Na^+ from the cytosol into the apoplast (Zhu, 2003).

All these responses can, in principle, contribute to plant salt tolerance. However, there is considerable variability regarding the efficiency and relative contribution of specific responses to tolerance mechanisms in a particular species. Therefore, the analysis of salt stress tolerance should be performed on a case-by-case basis, or at least studying closely related taxa, which could be assumed to use the same mechanisms, maybe differing only quantitatively in their degree of efficiency. Unfortunately, there is no single model, not even *Arabidopsis*, that could be used in these studies.

CONTROL OF K^+ HOMEOSTASIS UNDER SALT STRESS CONDITIONS

As mentioned above, high Na^+ concentrations interfere with the uptake of K^+ . Both cations have similar physicochemical properties and compete for the same binding sites in proteins, including K^+ membrane channels and transporters (Greenway & Munns, 1980; Flowers et al., 1986). In addition, Na^+ in excess causes depolarisation of the plasma membrane,

which in turn activates outward-rectifying K^+ channels, further reducing the cytosolic concentrations of the cation (Shabala et al., 2006; Shabala & Cuin, 2008). Therefore, an increase in cellular Na^+ concentrations is commonly accompanied by the simultaneous reduction of K^+ levels. Considering the fundamental role of the 'physiological cation' K^+ in photosynthesis, osmoregulation and turgor generation, regulation of membrane potential, essential cellular processes such as protein synthesis, and the activity of multiple enzymes, it should be assumed that the deleterious effects of salt stress are partly mediated by the reduction of cytosolic K^+ concentrations, especially in photosynthetic tissues (Almeida et al., 2017). Accordingly, this response has been reported in many salt-sensitive species (e.g., Isla & Aragüés, 2010; Tavakkoli et al., 2011), including the model plant *Arabidopsis thaliana* (Volkov et al., 2003).

Our studies have detected the same behaviour in different species with varying levels of salt tolerance. For example, in *Araujia sericifera* - an invasive plant that represents a problem in Southeast Spain as it causes damage to citrus orchards - the increase in leaf Na^+ and Cl^- concentrations with increasing external salinity correlated with the parallel reduction in K^+ contents; on the contrary, as should be expected, a water stress treatment did not modify leaf ion contents (Figure 1). The invasive potential of *A. sericifera* is increasing in the context of climate change, as it is relatively more salt tolerant than the affected native species. Nevertheless, in this case, tolerance mechanisms do not seem to depend on controlling K^+ homeostasis but rather on activating efficient antioxidant systems (Bellache et al., 2022).

Nerium oleander was another species studied in our laboratory to elucidate its salt tolerance mechanisms. This ornamental plant is grown in arid and semiarid regions because of its drought tolerance and seems to be also relatively tolerant to salinity. We could show that salt tolerance in oleander is primarily based on the accumulation of organic osmolytes (sugars and, to a lesser extent, glycine betaine), the activation of antioxidant systems and, at moderate salt concentrations, the inhibition of

toxic ions translocation to the leaves (Kumar et al., 2017). Control of potassium transport did not seem highly relevant for tolerance as K^+ contents showed a decreasing trend in response to increasing salinity in both roots and leaves, although this trend was more pronounced in the roots (Figure 2).

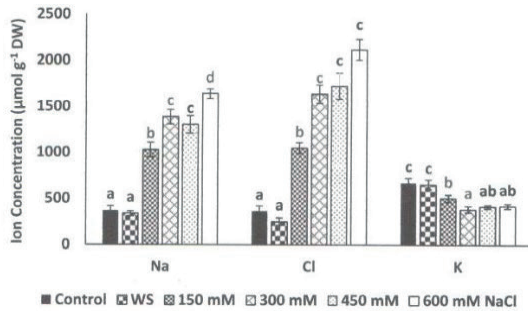


Figure 1. Sodium (Na), chloride (Cl), and potassium (K) leaf contents of *Araujia sericifera* plants after one month-treatments of water stress (WS, complete withholding of irrigation) and salt stress at the indicated NaCl concentrations. Means \pm SD, $n = 7$. Different lowercase letters above the bars indicate significant differences between the treatments for each ion according to the Tukey test ($\alpha = 0.05$) (Taken from Bellache et al., 2022)

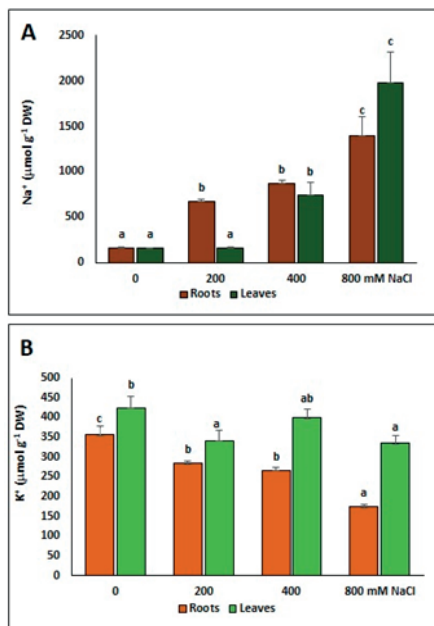


Figure 2. Monovalent ion contents, Na^+ (A) and K^+ (B), in roots and leaves of *Nerium oleander* after 30 days of salt stress treatments at the indicated NaCl concentrations. Values shown are means \pm SE ($n = 5$). Different letters above the bars indicate significant differences between the treatments for each organ, according to the Tukey test ($\alpha = 0.05$) (Adapted from Kumar et al., 2017)

Similar results were obtained in many other plants, including crops such as tomato

(*Solanum lycopersicum*) (Zuzunaga-Rosas et al., 2023). Also, in two Iberian endemic species of the genus *Gypsophila*, *G. struthium* subsp. *struthium* and *G. tomentosa*, which are closely related taxonomically and coexist in the same territory but have different ecological optima (Soriano et al., 2014).

SALT STRESS TOLERANCE BASED ON CELLULAR K^+ RETENTION

The patterns of changes in Na^+ (and Cl^-) contents in response to increasing salinity shown in the examples above, i.e., a parallel, concentration-dependent increase in roots and leaves, have been observed in most plant species. However, K^+ contents may vary widely in different species under the same conditions. Apart from the expected Na^+ -induced decrease in roots and leaves, we have observed different K^+ variation patterns in response to high salinity in salt-tolerant and sensitive species, remaining constant, continuously increasing, or even decreasing at low or moderate salt concentrations to increase again under stronger salt stress conditions. In all these cases, cytosolic K^+ retention (primary in leaf cells) should contribute to salt tolerance, to a greater or lesser extent depending on the presence of additional tolerance mechanisms. In the following sections, some specific examples will be presented, including forest species, ornamentals, crops, and wild taxa with different degrees and mechanisms of salt tolerance. In some cases, we have performed comparative analyses of salt stress responses in genetically related taxa, which allowed correlating the patterns of K^+ retention with the relative resistance of the selected plants - generally assessed by the relative salt-induced inhibition of plant growth - to support the physiological relevance of K^+ homeostasis in the mechanisms of salt tolerance (Vicente & Boscaiu, 2019).

FOREST TREES

There are few reports (e.g., Ran et al., 2021; Tikhomirova et al., 2023) on the responses of forest trees to salt stress, as their natural habitats are not saline - at least in temperate and cold zones of the northern hemisphere.

However, some of these species can be used in landscaping and grown as ornamentals in urban zones, gardens and city parks, where they can be irrigated with low-quality saline water. Also, the common winter practice of de-icing mountain roads with vast amounts of salt causes a high tree death rate every year (Fluckiger & Braun, 1981). Therefore, we have included some economically important forest trees in our studies on salt tolerance mechanisms.

Norway spruce (*Picea abies*). Growth inhibition is the best indicator of the adverse effects of stress in plants, including high salinity, but it is not easy to evaluate in species of slow growth, such as most forest trees. To identify biochemical salt stress markers in *P. abies*, we studied the responses to controlled salt treatments of young spruce seedlings obtained by germinating seeds with origin in seven different populations from the Carpathian Mountains in Romania. For all populations, as expected, Na^+ increased in roots and needles in parallel with the salt concentration in the irrigation water. On the other hand, K^+ contents decreased in the roots but increased significantly in the needles. This pattern suggested the activation in the presence of salt of efficient K^+ transport mechanisms from the roots to the aboveground organs, contributing to counteracting the deleterious effects of Na^+ in the needles (Schiop et al., 2015).

European larch (*Larix decidua*). The European larch is an important timber species, which also has great potential for landscaping, being one of the few deciduous coniferous species. As for Norway spruce, growth in the presence of salt led to the activation of K^+ transport to the aerial part of the seedling, as shown by the increase in the cation content in needles and its decrease in roots, in parallel with the increased levels of Na^+ in both organs (Figure 3). This mechanism appears to contribute significantly to the response of larch to high salinity, together with the accumulation of proline as a functional osmolyte (Plesa et al., 2018).

Silver fir (*Abies alba*). Silver fir is an economically and ecologically important forest tree species, especially in lower-mountain forests. One-year-old *A. alba* seedlings were subjected to a salt stress treatment with increasing concentrations of NaCl, up to 300

mM, and different growth parameters and biochemical stress markers were determined after 30 days. As shown in Figure 4, Na^+ contents increased progressively with increasing external salinity, following the same variation pattern in roots and needles.

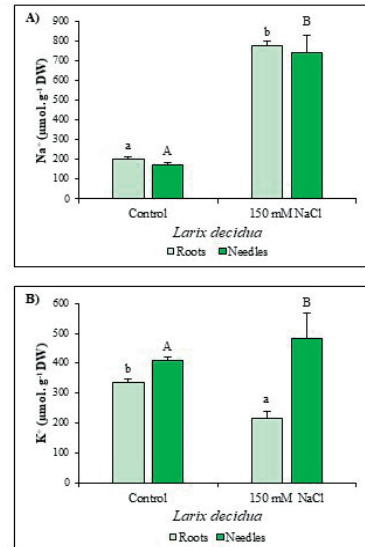


Figure 3. Monovalent ion contents, Na^+ (A) and K^+ (B), in roots and needles of *Larix decidua* seedlings after 30 days of salt stress treatments at 150 mM NaCl. Values shown are means \pm SE (n = 7). Different letters above the bars, lowercase for roots and uppercase for needles, indicate significant differences between the treatments, according to the Tukey test ($\alpha = 0.05$) (Adapted from Plesa et al., 2018)

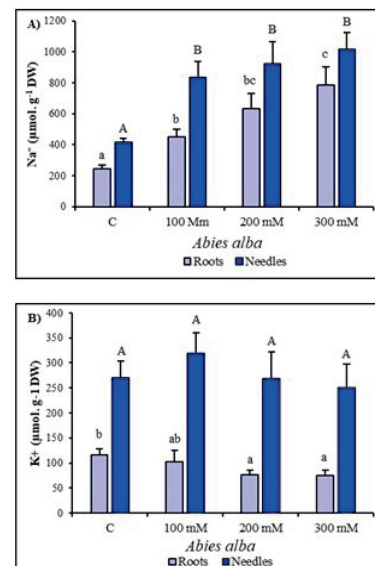


Figure 4. Ion contents, Na^+ (A) and K^+ (B), in roots and needles of *A. alba* seedlings after 30 days of growth in the presence of the indicated NaCl concentrations. Bars represent means \pm SE (n = 7). Different letters above the bars indicate significant differences between treatments, according to Tukey's test ($\alpha = 0.05$) (Adapted from Todea Morar et al., 2020)

On the other hand, K^+ concentration in the roots was significantly reduced compared to the non-stressed control in the presence of 200 and 300 mM NaCl but was maintained at the control level in the needles, suggesting the activation of mechanisms to avoid K^+ loss in photosynthetic tissue. As expected, Cl^- followed the same accumulation pattern as Na^+ , and Ca^{2+} also increased in both organs as a response to increasing salinity (Todea Morar et al., 2020).

ORNAMENTALS

Ornamental plant species with higher salt tolerance than our common food crops could represent profitable 'minor' crops to be commercially cultivated in salinized soils. Therefore, assessing their tolerance level and mechanisms is of practical interest as it would help select suitable genotypes for sustainable 'saline' agriculture. Apart from oleander, plants of other ornamental species have been investigated in our laboratory regarding their behaviour under high salinity (and/or drought) conditions. In *Calendula officinalis*, for example, the most effective responses to salt stress seemed to be mediated by the maintenance of K^+ (and Ca^{2+}) leaf contents with increasing salinity, up to 150 mM NaCl, together with the accumulation of proline as a functional osmolyte (Kozminska et al., 2017).

Lavandula angustifolia plants of two commercial varieties were also subjected to salt treatments (30 days, 100, 200 and 300 mM NaCl). The same patterns of K^+ contents variation in response to increasing salinity were found for both cultivars: a significant decreasing trend in roots and a slight but statistically significant increase in the leaves (Figure 5). The contents of all other measured ions, Na^+ , Cl^- and Ca^{2+} , rose in roots and leaves in parallel with increasing NaCl concentrations in the irrigation water. Here again, salt tolerance in these species primarily depends on maintaining K^+ (and Ca^{2+}) concentrations in salt-treated plants, apart from the synthesis and accumulation of proline for osmotic adjustment under stress (Szekely-Varga et al., 2020).

CROP SPECIES

Our common food crops are generally more sensitive to salinity and other abiotic stressors than their wild relatives. During the domestication process, traits related to rapid growth, biomass accumulation and yield were selected, resulting in the loss of efficient stress defence mechanisms (Kozioł et al., 2012; Østerberg et al., 2017).

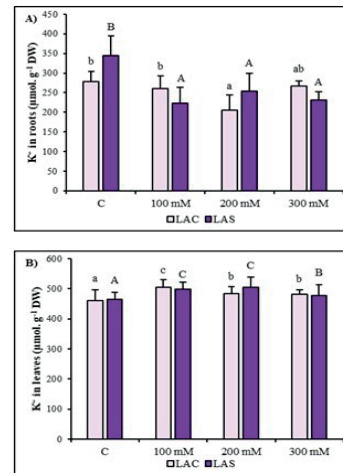


Figure 5. Potassium (K^+) contents in roots (A) and leaves (B) of *Lavandula angustifolia*, var. Codreanca (LAC) and var. Sevtopolis (LAS), after 30 days of growth in the presence of the indicated NaCl concentrations. Bars represent means \pm SE ($n = 5$). Different letters above the bars, lowercase for LAC and uppercase for LAS, indicate significant differences between treatments, according to the Tukey test ($\alpha = 0.05$) (Adapted from Szekely-Varga et al., 2020)

Nonetheless, salt stress responses, which can include the retention of K^+ in the cytosol of leaf cells, are also activated in crops. As an example, we briefly describe our studies on some bean species.

In initial experiments, we compared the responses to increasing NaCl concentrations of three commercial cultivars of *Phaseolus vulgaris*, the common bean - also including one of the runner bean (*P. coccineus*) - showing that salt tolerance is primarily based on the restriction of Na^+ (and, to a lesser extent, also of Cl^-) transport to the aerial part of the plants, as the most tolerant cultivar accumulated relatively lower concentrations of these ions in the leaves (Al Hassan et al., 2016c).

These results were confirmed using different *P. vulgaris* cultivars, four Spanish landraces and two experimental lines from Cuba (Figure 6). Again, the most tolerant genotype, identified by growth inhibition measurements (BGV015856), accumulated the lowest Na⁺ concentrations in the leaves. Interestingly, in all cases - except in the most sensitive E125 - leaf K⁺ contents increased in response to salt stress, especially at 150 mM NaCl, the highest concentrations tested, contributing to counteracting the toxic Na⁺ effects (Arteaga et al., 2020). The same results were obtained when analysing salt stress responses in four local *Phaseolus lunatus* varieties from Spain (Arteaga et al., 2018). In any case, considering the large number of cultivars of beans and other crops differing in their relative degree of salt tolerance, intraspecific quantitative differences should be expected in these general tolerance mechanisms.

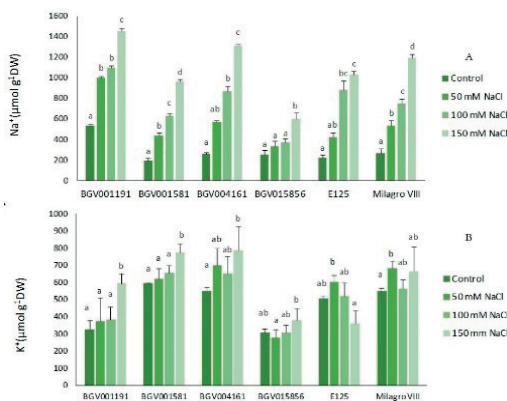


Figure 6. Monovalent cation, Na⁺ (A) and K⁺ (B) leaf contents in six common bean cultivars after 18 days of growth in the presence of the indicated NaCl concentrations. Values are means ± SE (n = 5). Different lowercase letters over the bars indicate significant differences between treatments for each cultivar, according to the Tukey test ($\alpha = 0.05$) (Taken from Arteaga et al., 2020)

HALOPHYTES

Halophytes are wild plants adapted to high soil salinity in their natural habitats and constitute the most appropriate systems to investigate salt tolerance mechanisms. In some species, morphological and anatomical traits - e.g., the presence of salt glands or succulence - contribute to salt tolerance (Grigore & Toma, 2017). Otherwise, all halophytes activate the same defence responses used by non-tolerant species, including control of ion homeostasis, synthesis of osmolytes and activation of

antioxidant systems. Regarding ion transport, salt tolerance in monocotyledonous halophytes seem to rely primarily on the inhibition of Na⁺ and Cl⁻ transport to the aboveground organs, as in glycophytes, whereas active transport of toxic ions to the shoots operate in dicotyledonous halophytes. The most tolerant species are succulent dicots, which can store large amounts of Na⁺ and Cl⁻ in their huge leaf cell vacuoles. The relevance and relative contribution to the salt tolerance mechanisms of all these responses, which may or may not include K⁺ retention in the leaf cells, vary widely in different halophytic species. This highlights the need to investigate a wide variety of halophytic taxa to get a comprehensive view of the strategies they use to cope with high soil salinity. Some examples of the work undertaken in our laboratory along this line are briefly described below.

Thalictrum maritimum is an endemic and endangered species in East Spain, sensitive to drought but relatively resistant to salt stress, behaving as a moderate halophyte. Salt tolerance in this species is partly based on the active transport of toxic ions to the shoots, as well as the accumulation of Ca²⁺ and the maintenance of K⁺ concentrations in the leaves, despite the increase of Na⁺ levels (Figure 7). This information will help design and implement conservation programmes for this threatened species (González-Orenga et al., 2020).

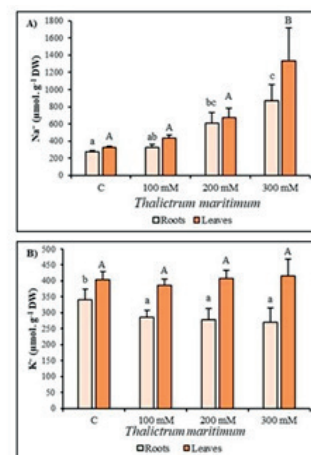


Figure 7. Ion contents, Na⁺ (A) and K⁺ (B), in roots and leaves of *Thalictrum maritimum* plants, after 23 days of salt stress treatments at the indicated NaCl concentrations. The values shown are means ± SE (n = 5). Different letters over the bars, lowercase for roots and uppercase for leaves, indicate significant differences between treatments, according to the Tukey test ($\alpha = 0.05$) (Adapted from González-Orenga et al., 2020)

Limonium is another interesting genus from a biodiversity perspective. It includes ~600 salt-tolerant (some also drought-tolerant) species, many of them endemics with different extensions of their geographic distribution areas. We have studied the responses to salt and water stress of several *Limonium* taxa, including some narrow endemics present in the Spanish Mediterranean region. Salt-tolerant *Limonium* species are recretohalophytes, which can secrete toxic ions through salt glands and salt bladders in their leaves. Apart from this anatomical adaptation, salt tolerance in this genus relies also on the transport and accumulation of toxic ions in the leaves, the concomitant use of different osmolytes to attain osmotic balance, and the activation of efficient antioxidant systems (González-Orenga et al., 2021 and references therein). However, cellular K^+ retention generally does not seem to contribute to salt tolerance, as in most analysed species, leaf K^+ concentrations decrease significantly with increasing salinity (Figure 8).

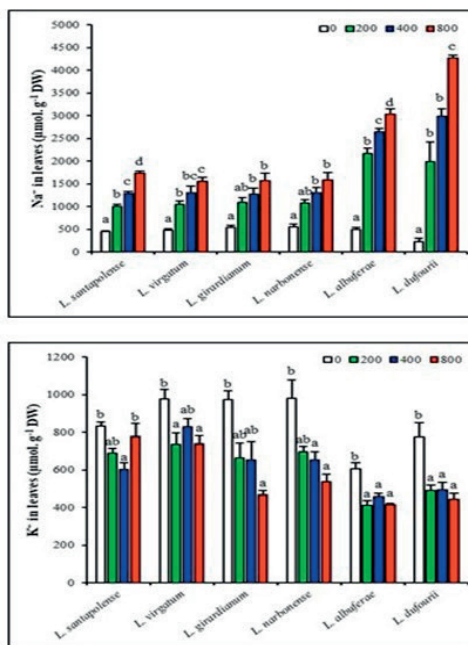


Figure 8. Accumulation of Na^+ (upper panel) and K^+ (lower panel) in foliar tissue of plants of different *Limonium* species, after one-month salt stress treatments at the NaCl concentrations indicated above (Adapted from González-Orenga et al., 2021)

However, *L. santapolense*, a local endemism present in salt marshes in the south of the Alicante province and highly salt tolerant, showed a somewhat different pattern. Mean leaf K^+ contents first decreased in parallel with

the increase in external salinity, as for the other *Limonium* species, but then rose to control levels at the highest salt concentration tested, 800 mM NaCl (Figure 8), clearly indicating that the activation of K^+ transport to the leaves is an important mechanism of tolerance to high salinity in *L. santapolense*. More recently, we have also detected a progressive increase in leaf K^+ contents in response to salt stress in *Limonium angustebracteatum*, another endemic species from the E and SE Iberian peninsula (Mir et al., 2022).

To investigate salt tolerance mechanisms in the genus *Plantago*, we carried out comparative analyses of the responses to salinity in three of its species, two halophytes, *P. crassifolia* and *P. coronopus*, and one glycophyte, *P. major*, growing the plants for one month in the presence of increasing NaCl concentrations (Figure 9). The tolerant species accumulated Na^+ (and Cl^-) in the leaves to higher concentrations than *P. major*, in agreement with the known fact that salt tolerance in dicotyledonous halophytes is based on the active transport of toxic ions to the aboveground organs, where they contribute to osmotic adjustment. In addition, proline levels strongly increased in response to very high salinity (600-800 mM NaCl) in the halophytes but not in *P. major* (Al Hassan et al., 2016d).

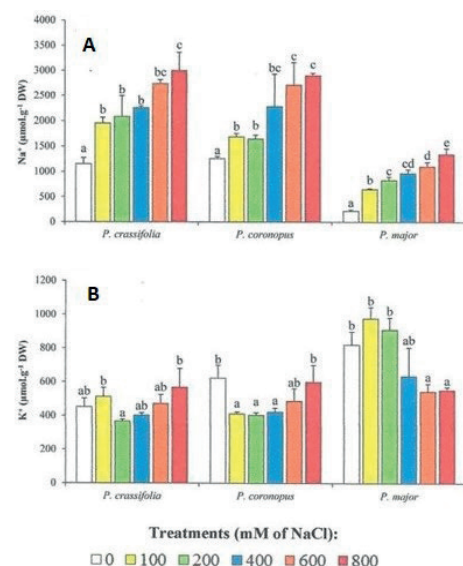


Figure 9. Leaf contents of monovalent ions, Na^+ (A) and K^+ (B), in plants of the selected *Plantago* species, after four weeks of treatment with the indicated NaCl concentrations (means \pm SD, $n = 5$). Different letters over the bars within each species indicate significant differences between treatments, according to the Tukey test ($\alpha = 0.05$) (Taken from Al Hassan et al., 2016d)

Regarding leaf K^+ contents, in *P. major*, they decreased significantly under high salinity conditions. In *P. crassifolia* and *P. coronopus*, on the contrary, mean K^+ levels decreased at moderate salinity to increase again, reaching control values in the presence of 600-800 mM NaCl (Figure 9). Therefore, activation of K^+ transport to the leaves seems to contribute substantially to the mechanisms of tolerance to high salinity in halophytes of this genus.

We also studied the responses to salt stress of three *Juncus* species - the halophytes *J. acutus* and *J. maritimus* and the glycophyte *J. articulatus*. In this case, as these species are monocots, salt tolerance partially depended on the inhibition of Na^+ and Cl^- transport to the shoots, with the salt-sensitive species *J. articulatus* accumulating higher concentrations of the toxic ions than the halophytes. A strong salt-induced accumulation of proline (~ 60-fold over control values) and the activation of K^+ transport to the shoots were observed in the presence of 400 mM NaCl in *J. acutus* and *J. maritimus* but not in *J. articulatus*, indicating that these specific responses also contribute to salt tolerance in the studied halophytes (Al Hassan et al., 2016b).

Dittrichia viscosa is a native-invasive species that has recently expanded into coastal salt marshes. To assess this species' potential risk to the original salt marsh vegetation, we compared its responses to controlled salt treatments in the greenhouse with those of *Inula crithmoides*, a closely related succulent halophyte. Also, the distribution of the two species in different salt marshes of 'La Albufera' Natural Park, close to the city of Valencia, was studied. Both species activated the same response mechanisms against salt stress, but they seemed to be more efficient in *I. crithmoides*, which accumulated Na^+ (and Cl^-), and osmolytes, mostly glycine betaine, to higher concentrations in the leaves than in *D. viscosa*. On the other hand, the level of oxidative stress under high salinity conditions was lower in *I. crithmoides* (Al Hassan et al., 2016a). In addition, leaf K^+ contents decreased under moderate salinity (300 mM NaCl) in the two species, increasing at higher salinities but much more strongly in *I. crithmoides*, reaching about 2-fold higher levels than in the non-stressed control in the presence of 600 mM NaCl (Figure 10). These

data explain the relatively higher salt tolerance of *I. crithmoides*, established by the relative salt-induced growth inhibition of both species and their distribution in their natural habitat. Therefore, *D. viscosa* cannot compete with *I. crithmoides* or other highly salt-tolerant halophytes in the salt marsh areas with the highest salinity. However, as *D. viscosa* is nonetheless quite salt-tolerant, it could represent a problem for the vegetation of less saline zones at the edges of the salt marsh, which includes many endemic and rare taxa of high ecological value in the area of this study (Al Hassan et al., 2016a).

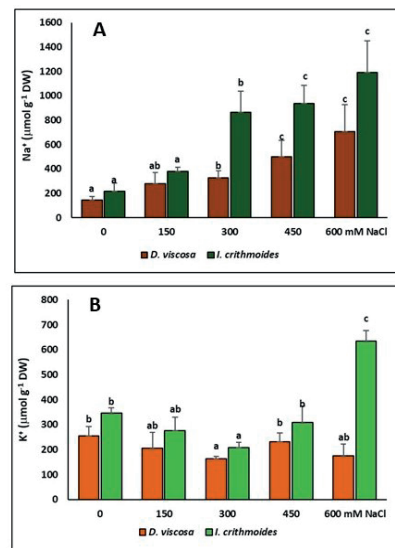


Figure 10. Leaf contents of sodium (Na^+) (A) and potassium (K^+) (B) in *Dittrichia viscosa* and *Inula crithmoides* plants after a 3-week treatment with the indicated salt concentrations. Values shown are means \pm SD (n=5). For each species, different letters over the bars indicate significant differences between treatments, according to the Tukey test ($\alpha = 0.05$) (Adapted from Al Hassan et al., 2016a)

CONCLUSIONS AND PERSPECTIVES

When analysing the mechanisms of salt tolerance based on the control of ion transport, special attention has been given to those involving Na^+ exclusion from the cytosol of photosynthetic cells, either by reducing Na^+ transport to the shoots or eliminating it from the cytoplasm by transport to the vacuole or the apoplast (Basil & Blumwald, 2014; Volkov, 2015; Zhu, 2003). Consequently, many attempts at genetic improvement of salt tolerance by classical breeding or genetic engineering have focussed on the control of

Na⁺ transport by the introgression or expression, respectively, of genes encoding specific transport proteins, such as HKT1; 1 and HKT1; 5 in roots (Møller et al., 2009; Munns et al., 2012) or the Na⁺/H⁺ antiporters of the plasma membrane, SOS1 (Shi et al., 2000) and the tonoplast, NHX1 (Apse et al., 1999).

It has also been emphasised that maintaining relatively low Na⁺/K⁺ ratios under high salinity conditions is critical for salt tolerance. However, this concept is misleading since what is relevant is the ratio in the cytosol, which generally represents only a small fraction of the cell, whereas toxic Na⁺ ions are primarily sequestered in the much larger vacuole volume. The Na⁺/K⁺ ratios are almost always calculated at the whole organ level, in roots, stems or leaves, due to the technical difficulties of measuring ions contents in subcellular compartments, as mentioned before. Therefore, absolute K⁺ concentrations, rather than Na⁺/K⁺ ratios, should be considered when analysing plant responses to salt treatments.

The examples included here, and many others in the literature, support the notion that cytosolic K⁺ retention is as important, if not more important, than Na⁺ exclusion for salt tolerance. Therefore, genetic improvement of this trait should focus more on manipulating K⁺ homeostasis through overexpression and tissue-specific expression of appropriate K⁺ transport proteins (e.g., Ali et al., 2018; Li et al., 2019; Shabala & Cuin, 2008).

ACKNOWLEDGEMENTS

We would like to thank all members of the group who, over the years, have contributed to the work described in this article. S.G-O acknowledges a 'Margarita Salas' postdoctoral contract from Universitat Politècnica de València and the Spanish Ministry of Universities, supported by the European Union - Next Generation funds.

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