Chapter 9
Roles and Transport of Sodium and Potassium in Plants

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Contents

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Abstract The two alkali cations Na^+ and K^+ have similar relative abundances in the earth crust but display very different distributions in the biosphere. In all living organisms, K^+ is the major inorganic cation in the cytoplasm, where its concentration (ca. 0.1 M) is usually several times higher than that of Na^+. Accumulation of Na^+ at high concentrations in the cytoplasm results in deleterious effects on cell metabolism, e.g., on photosynthetic activity in plants. Thus, Na^+ is compartmentalized outside the cytoplasm. In plants, it can be accumulated at high concentrations in vacuoles, where it is used as osmoticum. Na^+ is not an essential element in most plants, except in some halophytes. On the other hand, it can be a beneficial element, by replacing K^+ as vacuolar osmoticum for instance. In contrast, K^+ is an essential element. It is involved in electrical neutralization of inorganic and organic anions and macromolecules, pH homeostasis, control of membrane electrical potential, and the regulation of cell osmotic pressure. Through the latter function in plants, it plays a role in turgor-driven cell and organ movements. It is also involved in the activation of enzymes, protein synthesis, cell metabolism, and photosynthesis. Thus, plant growth requires large quantities of K^+ ions that are taken up by roots from the soil solution, and then distributed throughout the plant. The availability of K^+ ions in the soil solution, slowly released by soil particles and clays, is often limiting for optimal growth in most natural ecosystems. In contrast, due to natural salinity or irrigation with poor quality water, detrimental Na^+ concentrations, toxic for all crop species, are present in many soils, representing 6 % to 10 % of the earth's land area. Three families of ion channels (Shaker, TPK/KCO, and TPC) and 3 families of transporters (HAK, HKT, and CPA) have been identified so far as contributing to K^+ and Na^+ transport across the plasmalemma and internal membranes, with high or low ionic selectivity. In the model plant *Arabidopsis thaliana*, these families gather at least 70 members. Coordination of the activities of these systems, at the cell and whole plant levels, ensures plant K^+ nutrition, use of Na^+ as a beneficial element, and adaptation to saline conditions.

Keywords Channel • Enzyme • Membrane transport • Plant • Potassium • Sodium • Transporter • Turgor

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1 Introduction

K^+ is the most abundant cation in the cytosol, where the order of magnitude of its activity is 0.1 M, both in animals [1–3] and in plants [4–6]. The activity of Na^+ in the cytosol can display large variations, depending on the cell type, but is thought to usually remain lower than that of K^+ [3, 7, 8]. In plant cells, the cytosolic concentration of Na^+ seems to be controlled below 20–30 mM [9–11].

Reasons why K^+ has been selected as the major cytosolic cation during evolution and not Na^+, whereas the relative abundances of these two cations in the earth crust are quite similar, have been tentatively discussed [12, 13]. Life probably appeared in a seawater that possessed an ionic composition similar to that of the present oceans: a high concentration of Na^+, of a few hundreds of mM, and a much lower concentration of K^+, of a few tens of mM. It is thus possible that, for the first living cells, accumulation of the less abundant cation, K^+, and exclusion of the most abundant one, Na^+, was the simplest process to energize the cell membrane [13]. From a biophysical point of view, K^+ might have been selected during evolution because its hydration shell displays specific features, when compared to that of Na^+, in terms of hydration energy and hydration shell features. Weaker interactions with the first hydration shell in K^+ than in Na^+ results in larger structural flexibility and reduced disruption of the bulk water network and water arrangement close to proteins [14–16]. This might be the reason why Na^+ cannot totally replace K^+ as a coordinating ion in certain enzymatic reactions.

Molecular simulations aiming at investigating the physical and dynamical nature of the cytosol indicate that K^+ is essentially present as an unbound highly mobile osmolyte, populating the solution fairly uniformly and contributing thereby to control of the osmotic potential [17]. Na^+ can be used as osmoticum in plants, but essentially in the vacuole, where its concentration can become higher than 100 mM [18–20], and not in the cytosol [21].

With respect to the roles of Na^+ in the living world, the mechanisms underlying cell membrane energization and solute transport are fundamentally different between animals and plants. In animals, Na^+/K^+ ATPases energize the cell membrane by building up the Na^+ transmembrane electrochemical gradient that fuels Na^+-cotransporters and solute transport activity. In plants, the cell membrane is energized by H^+-ATPases (the so-called proton pumps), and active transport systems are H^+-cotransporters [22, 23]. There is so far no indication that the plasma membrane is equipped with transport systems using the Na^+ transmembrane electrochemical gradient to mediate active transport of solutes in higher plant cells. The only example of a Na^+-driven cotransport system identified in plants so far is a pyruvate transporter localized at the chloroplast envelope membrane [24, 25].

Another point that is worth to be noted in this introduction section is that most K^+ ions enter the trophic chain precisely at the moment when they are taken up by transport systems active at the plasmalemma of root cells. These transport systems are thus of major importance not only for plant K^+ nutrition and adaptation to low K^+ availability and but also for animal diet quality [26]. A major part of the present review is devoted to the presentation of the current knowledge on plant K^+ and Na^+ transport systems.

2 Potassium and Sodium Ion Concentrations in Soils

2.1 K^+ Availability in Soils

Potassium is amongst the 5 most abundant elements present in the upper continental crust, where its relative content, expressed in ppm, is close to 29×10^3 (2.9 %), a little bit above that of Na^+ (2.6 %). Therefore, soil K^+ reserves are generally large. However, important agricultural areas of the World are reported to be deficient in K^+ availability. This is the case for instance of 3/4 of the paddy soils of China, and 2/3 of the wheat belt of Southern Australia [27–29].

Three types of K^+ pools can be operationally distinguished in the soil [26]. Most of the K^+ ions in soil are in the so-called "structural form" (Figure 1), mainly comprised of K^+-bearing primary minerals such as muscovite, biotite, and feldspars. This first pool of K^+ ions is considered as slowly- or non-available to plants. However, it contributes to the plant supply in the long term [30, 31]. The other two pools contribute directly to plant nutrition [32–34]. The second pool, in terms of pool size, is constituted by potassium ions fixed in 2:1 interlayer clay minerals and is called the "non-exchangeable'" potassium pool (Figure 1) [35]. The size of this "non-exchangeable" pool depends on the soil content in clays. Illite and illite-like clays are the major sources of K^+ at least in temperate region soils [36]. The third pool is usually operationally defined as potassium ions readily "extractable" by water and/or aqueous solutions (Figure 1). Simply speaking, it comprises the pool of K^+ ions either dissolved in the soil solution or weakly bound to soil minerals. Upon K^+ concentration decreases in the soil solution, due to, e.g., root K^+ uptake, chemical equilibria result in desorption of K^+ ions from the pool of weakly bound exchangeable ions as well as in K^+ release from the "non-exchangeable" pool and even from K-feldspars [28].

The concentration of K^+ in soil solution is highly variable. Typical concentrations lie in the 10^{-5} to 10^{-3} M range [37–39]. It should be noted that, when compared with root K^+ uptake capacity, K^+ diffusion in the soil can be rate-limiting. In other words, roots are able to take up K^+ at a higher rate than this cation can diffuse from the bulk soil solution to the root surface, at least in some soils and environmental conditions. This results in K^+ depletion at the root surface, down to very low concentrations, of a few μM [40–42].

2.2 Plant K^+ Demand and K^+ Deficiency

Plant K^+ contents for optimal growth are in the range of 2–5 % of the dry weight of vegetative parts [35, 38]. In the case of maize or wheat plants grown in fertilized field conditions, K^+ uptakes of about 200 kg per hectare (ha) have been reported [43, 44]. The amount of K^+ actually removed from the field depends of course upon the plant parts that are harvested. For instance, more K^+ is removed when almost all

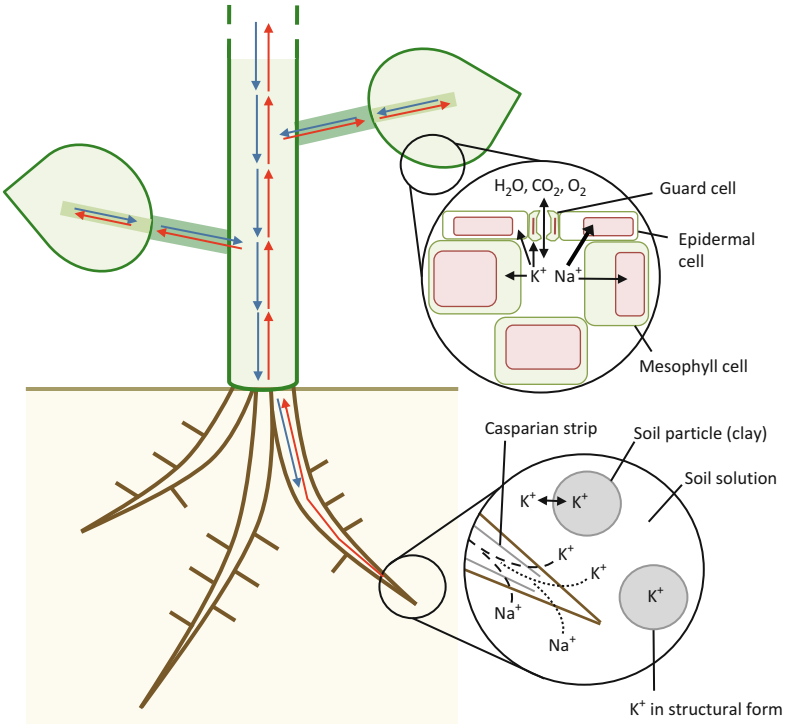

Figure 1 Overview of K^+ and Na^+ uptake and long distance transport between roots and shoots. Three K^+ pools can be operationally identified in the soil: (i) K^+ in "structural form" (slowly- or non-available to plants), (ii) "non-exchangeable" K^+, weakly bound at the surface of clay minerals, and (iii) extractable K^+ in aqueous soil solution. In root differentiated zones, due to the presence of the endodermis Casparian strip (solid gray line) that acts as a barrier preventing free diffusion within the so-called free space (cell wall continuum) towards the center of the root, K^+ and Na^+ ions (as well as other nutrient ions) have first to be selectively taken up by plasma membrane transport systems of root peripheral cells (epidermis, cortex, and endodermis) to enter the root symplasm. Then, diffusion within the root symplasm allows ions to pass the endodermis barrier (dashed line pathway) and to reach the root stele and xylem vasculature, located at the center of the root, where the flow of crude sap carries nutrient ions towards the shoots. The apical (meristematic) region of the growing root does not possess such an endodermis barrier. In these zones, ions can freely diffuse within the apoplasm and directly reach the root stele and xylem vessels (dotted line pathway), without any control by membrane transport systems. Once in the xylem, ions are transported (mass flow) towards and throughout the shoots by the xylem sap flow (red arrows). Arrived in leaves and unloaded from leaf vascular tissues, K^+ and Na^+ reach the leaf apoplasm, from which they are taken up by mesophyll, epidermal and guard cells. Na^+ ions are preferentially accumulated in leaf cell vacuoles and selectively compartmentalized in epidermal cells (wider arrow). Rapid K^+ transport into and out of guard cells controls the turgor of these cells, and thereby the diameter of the stomatal pore and the gas exchange rates between the inner leaf tissues and the atmosphere. In mature photosynthesizing (source) leaves, K^+ and Na^+ ions can also be loaded into the phloem sap (blue arrows) to be transported towards sink organs (roots, young leaves, fruits, etc.).

aboveground biomass is taken off at harvest (like in the case of sugar cane) than when only the seeds (cereals) are harvested [45]. In intensive agricultural production systems, lack of sufficient K^+ fertilizer application leads to significant depletion of available soil K^+ reserves [46]. A considerable area of farmland has become K^+-deficient [47–49]. Early visible symptoms of K^+ deficiency are brown spots at leaf surface or leaf edge and chlorosis at the tip of the oldest leaves. Severe K^+ deficiency results in further symptoms, including wilting and necrosis [26].

2.3 *Saline Soils, Na^+ Toxicity, and Plant Adaptation to Salt Stress*

Soil salinity results in both reduced soil water availability (due to the decrease in water potential) and ionic toxicity. Most crop plants are sensitive to soil salinity, and evidence is available at the physiological and molecular levels that Na^+ is the major cause of the toxicity in most cases. Indeed, for instance, genetic analyses and searches for QTLs of plant tolerance to salt stress have identified genes encoding Na^+ transporters or channels involved in Na^+ entry into the root or control of Na^+ transport to shoots in the model plant *Arabidopsis thaliana* [50] as well as in rice and wheat [51–53]. It should however be noted that, at high concentrations, the anion Cl^- can display its own toxicity [54].

Soils are classified as saline when their salt content affects the growth of most crop species. This may occur when the conductance of the soil solution (at soil saturation water content) displays an electrical conductance corresponding to that of 50 mM NaCl solution [38]. However, the impact of a given amount of salts on plant growth depends on the plant species, climatic conditions, soil features such as the water capacity and texture, and the nature of the salts, e.g., NaCl or Na_2SO_4, the former salt being more toxic than the latter in most species [55]. Estimates of the area of salt-affected soils vary widely, ranging from 6 % to 10 % of the earth's land area [52, 56]. Amongst "natural" saline areas are salt marshes of the temperate zones, mangrove swamps of the subtropics and tropics, and lands with saline underground water in arid and semi-arid regions where evapotranspiration is high. Besides such natural contexts, soil salinity can result from intensive agriculture and irrigation with low quality water [57]. This salinization process, which already affects 20 % of the irrigated lands, would result in loss of 3 ha of arable land from conventional crop farming every minute [58]. Enhanced demand for irrigation due to both population increase and climate change, are predicted to dramatically speed up such a loss of arable land [57, 59].

Plant adaptation to salt stress (resulting from high external Na^+ concentrations) is one of the most widely investigated domains in plant biology [52, 58, 60]. Large differences in tolerance level have been observed amongst species and even cultivars or ecotypes. At one extreme are plants named glycophytes, like the model plant *Arabidopsis thaliana*, rice or bean, which are sensitive to salt stress, being strongly

affected by external NaCl concentrations higher than about 50 mM. This is the case of most plant species and crops [52]. At the other extreme are plants named halophytes, like *Aeluropus littoralis* or the saltbush Atriplex, which can thrive in environments where the salt concentration is higher than 300 mM [52, 61]. Such species constitute about 1 % of the world's flora [62]. In between are species such as barley or alfalfa, which can cope with moderate salt concentrations [52, 63].

The mechanisms involved in tolerance to salt stress are highly complex and variable amongst species. They include, for instance, biochemical responses leading to synthesis of large amounts of organic osmoprotectant molecules, such as proline or glycine betaine, or anatomical adaptations such as the presence at the leaf surface of salt glands that excrete NaCl, or of very large cells, named salt bladders, expanding from the leaf epidermis and sequestering excessive Na^+ away from internal (photosynthesizing) leaf tissues [64] (see also below, Section 3.2). Despite this large diversity of mechanisms, in every species and every soil conditions, glycophytes in the presence of low external salt concentrations or halophytes in the presence of high salt concentrations, adaptation to external Na^+ involves tight regulation of K^+ and Na^+ membrane transport activity, especially allowing selective K^+ uptake by roots, control of Na^+ uptake and translocation towards the shoots by the xylem sap, and efficient Na^+ compartmentalization into vacuoles. It is also worth noting that part of the toxic effects of Na^+ is likely to lie in disruption of the K^+ membrane transport [65, 66] and homeostasis [10].

3 Potassium and Sodium Ion Fluxes and Distribution Within the Plant

3.1 K^+ and Na^+ Uptake and Long Distance Transport Between Roots and Shoots

Plant roots exhibit a polarized anatomy in which outer layers (epidermal and cortical cells) are involved in K^+ (and other nutrient ions) selective uptake and inner layers (endodermis and central vasculature) in secretion of the nutrient ions into the xylem sap towards shoot tissues [67]. In plants, an external skeleton named cell wall, essentially made of cellulose, hemicellulose, and polygalacturonic acids, forms a matrix freely accessible to water and soluble ions that surrounds the plasma membrane of each cell. The cell wall continuum is named "free space" or apoplasm. In the root, nutrient ions from the soil can enter the apoplasm and freely diffuse towards inner cell layers. However, this pathway is interrupted by a cell layer, named endodermis, whose radial cell walls are impregnated with hydrophobic compounds, forming the so-called Casparian strip, which is impermeable to water and ions [38, 68, 69]. Thus, the endodermis is the dead-end of the apoplastic pathway [70, 71]. To go beyond this impermeable barrier towards the center of the root and the plant vasculature, nutrient ions have to enter the cytosol of a root peripheral cell

(from the epidermis, cortex or endodermis). They can then diffuse from cell to cell through pores, named plasmodesmata, which connect the cytosolic milieu of two neighboring cells [72]. Diffusion within the symplasm beyond the endodermis barrier allows nutrient ions to reach the central part of the root, named stele. Once in the stele, nutrient ions have to be secreted into the stelar apoplasm, where they diffuse towards the xylem vessels, which contain the so-called crude (or xylem) sap. The sap flows upward towards the shoots (mass flow pulled by the transpiration stream or pushed by the so-called root pressure), providing the aerial parts of the plant with nutrient ions.

The Casparian strip is always absent in the apical (meristematic) region of the root and temporarily absent in the differentiated part of the root at points of secondary root emergence (Figure 1) [73]. At the level of such defects in the endodermis barrier, ions can freely diffuse within the apoplasm and reach the root stele and xylem vessels. However, this uncontrolled flow of nutrient ions towards the xylem sap remains relatively low. Most of the ions that reach the root xylem vessels have been taken up across the plasma membrane of a root peripheral cell at least one time [69]. In other words, their entry into the root symplasm has been "catalyzed", and thus controlled, by membrane transport systems, channels, transporters or co-transporters. Such a control of ion translocation from the soil to the shoots within the roots concerns all nutrient ions, including of course K^+ and Na^+, under standard physiological conditions [66, 74–76]. However, in saline soils, where the concentration of Na^+ is high, an important amount of this cation can reach the stele and xylem vessels directly through the defects in the endodermis barrier, without any control by any cell membrane. Such an uncontrolled flow of Na^+ ions contributes to the stressing effects of the external saline conditions [73, 77].

Once in the root xylem vasculature, during their mass flow-mediated transport towards the shoots (Figure 1), K^+ and Na^+ ions can be reabsorbed from the xylem sap into adjacent cells (parenchyma cells). This reabsorption is likely to result in increased root K^+ contents and thereby to favor root growth (at the expense of shoot growth) upon abiotic stresses such as drought or K^+ deficiency [78–81]. Regarding Na^+, retrieval of this cation from the xylem sap (the so-called sap-desalinization process) results in reduced Na^+ translocation towards the shoots and photosynthesizing tissues, which contributes to plant adaptation to a salinity constraint [50, 64, 82–84].

When arrived in leaves via the xylem vasculature, K^+ and Na^+ are used by various cell types. The photosynthesizing mesophyll cells, which constitute the largest part of leaf internal tissues, accumulate K^+ and Na^+ from the apoplastic and the symplastic pathways (Figure 1). Symplastic connections between mesophyll cells and epidermal cells seem to be transitory [85–87]. In the epidermis, the guard cells (osmocontractile cells that control the apertures of the stomatal pores; see below Section 4.2) are symplastically isolated. Thus, ion fluxes underlying their osmocontractility (K^+ playing the major role in this process) occur through specialized membrane proteins (essentially Shaker channels for K^+ [88, 89]; see Sections 4.2 and 5). It is worth noting that the leaf apoplasm volume is relatively small, so that rapid arrival of Na^+ ions upon saline conditions, if these ions are not rapidly taken up by leaf cells (and compartmented in the vacuoles, see below), can result in progressive

increase in Na^+ concentration in the cell walls. This leads to a decrease in external water potential down to values lower than the water potential in the cell, resulting in water efflux from the cells, loss of turgor and eventually cell death.

Evidence is available that Na^+ ions are preferentially accumulated in epidermal cells (Figure 1). This compartmentalization process, at the tissue level, protects the inner photosynthesizing mesophyll cells against detrimental effects of high Na^+ concentrations. At the whole plant level, Na^+ can be preferentially accumulated in old leaves to prevent toxic accumulation in the younger ones [90]. Na^+ ions can also be re-circulated towards the shoots by the elaborated sap flow [50]. This sap, which is produced in leaves, contains photosynthates (mainly sucrose). It flows (osmotically driven mass flow) within the phloem vasculature towards sink organs (roots, young developing leaves, fruits, etc.) that it feeds. The phloem tubes, which conduct this elaborated sap (more often named phloem sap) are living cells that do not display the large central vacuole typical of many plant cell types. As living cells, they display the classical ionic composition of the cytosol, i.e., low Na^+ concentration and high K^+ concentration. Probably related to that, the rate of Na^+ recirculation from shoots to roots by the phloem sap [50] is relatively low when compared to that of K^+ [73, 91, 92]. Regarding the latter ion, it has been reported that K^+ recirculation from shoots to roots by the phloem sap can provide, at the steady state, more than 60 % of the amounts of K^+ ions transported from roots to shoots by the xylem sap, the difference corresponding to the actual uptake of K^+ from the soil solution (Figure 1) [93, 94]. This large and continuous cycling of K^+ ions from roots to shoots via the xylem vasculature and from shoots to roots via the phloem vasculature would play a role in K^+ demand signaling and regulation of K^+ uptake and distribution [79, 91, 95, 96].

3.2 Cellular Compartmentalization of K^+ and Na^+

As indicated above, with the exception of some cell types such as phloem tube cells and meristematic cells, most plant cells display a large central vacuole, which represents approximately 90 % of the cellular volume and can thereby be the main deposit for K^+ and Na^+ ions within the cell [97, 98]. K^+ accumulation in the vacuole leads to turgor build-up that importantly contributes to cellular expansion and/or osmocontractility and cell movements (see Section 4.1). The concentration of K^+ in the vacuole can also substantially vary depending on availability of this cation in the nutritive solution [6, 9, 38, 99]. Upon K^+ starvation, vacuolar K^+ ions are released into the cytoplasm, allowing a relative homeostatic control of the cytosolic K^+ concentration, close to 0.1 M [4]. Once the vacuolar K^+ pool is exhausted, the cytosolic concentration of K^+ gradually decreases [6, 100], resulting in detrimental effects on cell physiology and eventually death.

Both the plasma membrane and the vacuolar membrane (also named tonoplast) are energized by transmembrane electrochemical H^+ gradients built up by proton pumps, which are P-type H^+-ATPases in the case of the former membrane, and V-type ATPases and H^+-secreting membrane pyrophosphatases in the case of the

latter one. The electrical component of the gradient across the plasma membrane can be very negative (–250 mV; negative inside) and variable, depending on H^+ secretion activity but also on K^+ external concentration (see Section 4.3) [101–103]. The magnitude of the transmembrane electrical gradient across the tonoplast is much less important, being at most of a few tens of mV, the vacuolar lumen being positive with respect to the cytosol [6, 91, 104]. K^+ and Na^+ fluxes are mediated by channels and uniporters, and active fluxes mediated by H^+-cotransporters (symporters or antiporters) [23].

Regarding K^+, strong membrane hyperpolarization of the plasma membrane allows efficient uptake of this cation through K^+ channels even from diluted external solutions, displaying K^+ concentrations as low as about 10 μM [23, 102, 105, 106]. H^+:K^+ symporters are responsible for active K^+ uptake from still more diluted solutions [23, 103, 107]. In some physiological situations and cell types (e.g., in xylem parenchyma cells bordering the xylem vessels and responsible for nutrient ion secretion into the xylem sap (see above), or in guard cells during stomatal closure (see below, Section 4.1)), the plasma membrane can become poorly polarized, allowing K^+ efflux through K^+ channels [78, 108–110].

Regarding Na^+, vacuolar accumulation of this cation decreases the cellular water potential and thus contributes to cell turgor (see below Section 4.1). It can thereby have beneficial effects on plant growth, especially when K^+ availability is low. However, since Na^+ is toxic when largely accumulated in the cytosol, plant cells try to minimize the cytosolic pool of this cation when its external concentration becomes high [110]. Such a control is critical for preventing toxicity symptoms, which mainly stem from Na^+ effects on cellular metabolism [111] (see Section 4.4). Plants prevent large accumulation of Na^+ in the cytosol by compartmentalizing this cation into the vacuole [73] or extruding it outside the cell, e.g., in soil in the case of root peripheral cells, or at the leaf surface, and not into the leaf apoplast where increased concentrations of Na^+ would have detrimental effects (see above). Some halophytes (salt-adapted species) like *Distichlis spicata* or mangroves produce exudates that are highly concentrated in Na^+, from leaf specialized structures called salt glands, which result from the modification of hydathodes [73, 112, 113]. Other species deposit significant amounts of Na^+ in either swollen vacuoles in succulent tissues or in specialized external structures called epidermal bladder cells [58, 62]. Such salt bladders seem to originate from trichomes in which the outmost cell grows until it takes a balloon-like form with a huge central vacuole [114, 115].

4 Roles of Potassium and Sodium Ions in Plants

4.1 Roles of K^+ in Cell Turgor Building

Accumulation of solutes like K^+ or Na^+ inside the cell lowers the water potential, leading to water entry into the cell. This can have beneficial effects under adverse conditions like drought in which K^+ accumulation within plant cells gives rise to an

improved osmotic adjustment. In case of Na^+ excess, keeping high internal K^+ concentrations has been proven to be a key determinant of salt tolerance by helping retaining water and reducing Na^+ uptake [110, 116].

Since the cell wall restricts changes in cell volume, water uptake due to solute accumulation results in hydrostatic pressure (turgor). Such pressure is the primary force driving cell growth through cell expansion, and thereby growth at the tissue and whole plant levels. Fruit growth provides a typical example of turgor-driven cell expansion where flesh cells accumulate K^+, solutes and water to increase in size. On the other hand, cell expansion also requires changes in the cell wall architecture in order to be extensible. This involves loosening and the continued cutting and pasting of new material into the texture of the wall [117].

4.2 Role of K^+ in Turgor-Driven Movements in Plants

4.2.1 Regulation of Stomatal Aperture at the Leaf Surface and Control of Gas Exchanges with the Atmosphere

In terrestrial plants, a protecting waxy cuticle covers the epidermis of the aerial organs, preventing water loss and desiccation. Simultaneously, this hydrophobic barrier impedes diffusion of atmospheric CO_2 towards the inner photosynthesizing tissues. Gas exchanges between these tissues and the atmosphere mainly take place through microscopic pores, named stomata (Figure 1). Two osmocontractile cells surrounding the pore, named guard cells, control stomatal aperture. Such a control allows the plant to cope with the conflicting needs of maintaining a sufficient internal CO_2 concentration for photosynthesis and of preventing excessive transpirational water loss under diverse environmental conditions [118, 119]. Guard cells regulate the aperture of stomatal pores in response to many physiological stimuli such as light, soil water availability and leaf water status, CO_2 internal concentration and hormones [120, 121]. These cells are not connected to the symplasm of their neighboring cells (via plasmodesmata) and their cellular movements are rapid and driven by osmotic changes. Stomatal pore size will determine gas exchange rates between photosynthetic cells and the atmosphere. An increase in guard cell turgor promotes pore opening, whereas a decrease in turgor leads to stomatal closure [122].

The available information indicates that the main solutes involved in the guard cell osmocontractility are K^+, accompanying anions (malate and chloride) and sucrose, depending on the environmental conditions. During stomatal opening, guard cell volume significantly increases because of the activation of plasma membrane H^+-ATPases and inwardly-rectifying K^+ channels from the Shaker family (see Section 5.1) and organic acid production and uptake of inorganic anions (mainly Cl^- and NO_3^-) [121, 123]. Osmolyte accumulation leads to a lower water potential in guard cells, which, in turn, induces osmotic water influx into these cells. Moreover, multiple smaller vacuoles fuse to form a large central vacuole that leads to a remark-

able increase in guard cell volume [124]. K^+ uptake into vacuoles during stomatal opening is dependent on H^+/K^+ antiporter activity [125, 126]. During stomatal closure, guard cell volume decreases owing to net cellular efflux of solutes. Anion efflux through anion channels induces membrane depolarization that activates outwardly-rectifying K^+ channels, leading to K^+ efflux [127, 128]. The resulting cellular export of K^+, Cl^- and organic ions results in water efflux from guard cells. At the vacuole membrane, Ca^{2+}-activated K^+ channels are involved in K^+ release into the cytoplasm [118, 129].

4.2.2 Leaf Movements and Other Organ Movements

Plants also display organ movements, which are classically sorted into two main types, tropic and nastic movements. Tropisms are oriented by the direction of the stimulus that induce the movement. In contrast, the direction of a nastic movement is independent of the stimulus's direction or position. Another difference is that the movement is irreversible in the case of tropisms, while it is generally reversible and repeatable in the case of nastic movements. The distinction between these two types of movement is however sometimes unclear.

A tropism (from Greek, tropos, to turn) relies on asymmetric growth between two opposite regions of the organ due to redistribution of growth regulators, coordinated cell division and turgor-dependent cell expansion. It also involves K^+ transport and accumulation. The difference in growth results in a curvature of the growing organ towards or away from the stimulus (e.g., light or gravity). A classical example of such a movement is root gravitropism [130, 131]. Although most nastic movements do not involve growth responses, it is worth to note that the terms epinasty and hyponasty are used to qualify bending of an organ which does involve differential growth, e.g. greater growth of the upper side than of the lower side of the leaves in plants displaying leaf epinasty [132, 133].

Other nastic movements, which are reversible, result from a gradient of turgor between two opposite regions of the organ. The direction of movement is thus determined by the anatomy of the organ rather than by the stimulus. For example, the pulvinus, a joint-like group of differentiated motor cells at the base of the petiole, is responsible for nyctinastic leaf movements in plants that can orientate their leaves to a vertical position during the dark period. A difference in turgor between cells on one side of the pulvinus, behaving as extensors, and cells on the opposite side, behaving as flexors, controls leaf angle. In a sensitive plant, seismonastic movements, by which leaves respond to mechanical stimuli, also involve gradients of cell turgor in the pulvinus. In such movements, turgor changes are triggered when K^+ ions (and accompanying anions) move into or out of the cells, and water follows by osmosis (like in guard cells during the opening and closing of stomata; see above). Voltage-gated inwardly- or outwardly-rectifying K^+ channels belonging to the Shaker family (see below, Section 5) mediate the K^+ influxes and effluxes, respectively, that underlie the changes in turgor [134, 135].

4.3 Role of K^+ in Control of the Cell Membrane Potential

K^+ concentrations at both sides of the plasma membrane have a great impact on the polarization of cell membrane potential. Electrophysiological analyses indicate that the resting plasma membrane potentials significantly vary in response to changes in K^+ external concentrations [101, 103]. K^+ channels from the Shaker family have been shown to be responsible for a large part of these variations in many cell types and physiological conditions [102, 136]. The dependency of the cell membrane potential on external and internal K^+ concentrations indirectly affect the transport of other solutes that rely on cell polarization to enter or exit the plant cell.

K^+ is also involved in electrical signals (action potentials) that plants are able to generate in response to different stimuli (cold, wounding, etc.). Unlike in neurons and other animal cells, in which Na^+ is a crucial player in action potentials (an inward flow of Na^+ ions triggering a depolarization of the cell membrane, which is thereafter rapidly repolarized by an outward flow of K^+ ions), excitation in plant cells mainly involves an efflux of anions (Cl^- or NO_3^-) for the initial depolarizing event. Then, the membrane depolarization gives rise to a repolarizing efflux of K^+ ions, like in animal cells [137]. For instance, the electrical signal allowing rhizobacteria recognition by leguminous roots, after perception of the bacterial Nod factors, would require K^+ efflux to repolarize the plasma membrane after a Cl^--induced membrane depolarization [138].

4.4 Effects of K^+ on Enzyme Activities and Roles in Metabolism

Since the initial reports that pyruvate kinase (PK) activity is strongly stimulated by K^+ [139, 140], many enzymes have been identified as being activated by monovalent cations in animals, bacteria and plants [141–143]. Activation involves selective binding of the monovalent cation to the enzyme or enzyme-substrate complex, stabilizing catalytic intermediates and enzyme structure, or providing optimal positioning of substrate. Molecular determinants of the binding of the monovalent cation to the protein have been identified in several enzymes [142], including from plants [144, 145]. The activation process involves selective interactions between the monovalent cation and the enzyme. The activating cation is most often K^+. This is the case, for instance, in various enzymes catalyzing phosphorylation of a carboxyl group or enolate anion and of molecular chaperones [142, 143]. Fewer enzymes, such as galactosidase and clotting proteases, are selective for Na^+ [142]. When activated by K^+, the enzymes are usually also significantly activated by Rb^+ and NH_4^+, while they are weakly activated by Na^+ and often not at all by Li^+ [141–143]. For instance, in plants, the starch synthetase from sweet corn displays an absolute requirement for potassium, with the optimum activation occurring at 50 mM KCI. Rb^+, Cs^+, and NH_4^+ are 80 % as effective as K^+, while Na^+ and Li^+ are

respectively 21 % and 8 % as effective [146]. Conversely, enzymes displaying selectivity for Na^+ are also sensitive to Li^+, and much less to K^+, Rb^+ or NH_4^+ [141]. The mechanisms underlying the ionic selectivity are not always fully understood. For instance, in pyruvate kinase, replacement of K^+ with Na^+ does not seem to result in any apparent structural change [147], although the enzyme is practically inactive without K^+.

In plants, key enzymes (including membrane transport systems), like glutamine synthetase, phosphoenolpyruvate carboxylase (PEPC), phosphofructokinase, ADP-glucose starch synthase and some vacuolar PPases (involved in H^+ secretion across the vacuolar membrane into the vacuole) are strongly activated by K^+ [38, 141, 148, 149]. More generally, protein synthesis requires high concentrations of K^+ [4, 150]. It is worth noting that this biochemical requirement of protein synthesis for K^+ has often been taken as indirect evidence for strong homeostatic K^+ control in the cytosol [151]. Plant K^+ status can also affect plant metabolism through transcriptional and post-transcriptional regulation of metabolic enzymes. For instance, transcriptome analyses in K^+-starved *A. thaliana* plants have provided evidence for upregulation of malic enzyme and the GS/GOGAT cycle and downregulation of nitrate uptake and reduction [152, 153]. Other widely reported consequences of K^+-deficiency are accumulation of reducing sugars and depletion of organic acids and negatively charged amino acids [150].

Hence, a large set of data provides evidence that K^+ availability strongly affect plant contents of primary and secondary metabolites [150, 154]. As discussed in [153], it seems likely that at least part of such changes in metabolite contents reflects direct responses of enzyme activities and metabolism to the internal concentration of K^+, although this concentration is homeostatically controlled *in vivo* and thus poorly sensitive to large variations in the availability of K^+ in the external medium.

4.5 Roles of Na^+ in Plants

4.5.1 Replacement of K^+ by Na^+ as Vacuolar Osmoticum

K^+ is an essential macronutrient for plants because there are specific cellular functions that only K^+ can meet (see above). On the other hand, the role of K^+ as osmoticum in the vacuole is non-specific. In this function, this cation can be replaced by other solutes, and in particular by Na^+ [38]. Quantitatively, the K^+ cytosolic pool can represent a small fraction of the total amount of K^+ ions within a plant cell when the availability of this cation in the external medium (nutritive or soil solution) is not limiting. In such conditions, most of the cellular K^+ (around 90 %) is stored in the vacuole for osmotic purposes [155, 156]. However, when the availability of external K^+ is low, Na^+ can be substituted for K^+ as osmoticum in the vacuole, so that a relatively small amount of K^+ is sufficient to maintain K^+-specific functions in the cytosol. Indeed, it has been shown that Arabidopsis plants exhibited higher growth rates if 10 mM Na^+ was added to a 10 μM K^+ growth solution [107]. In another

example, Na^+ uptake in rice plants proved to be crucial for biomass production when K^+ was limiting [157].

4.5.2 Na^+ Is a Beneficial Nutrient

Na^+ does not seem to be an essential nutrient in most higher plants (in all plants displaying C3 photosynthetic pathway, like the model plant *Arabidopsis thaliana*, and part of the plants displaying the C4 pathway, like maize and sorghum) [10]. However, because Na^+ stimulates growth in many plant species and can partly replace K^+ in some functions [39, 158–160] like osmotic adjustment of the large central vacuole, cell turgor regulation allowing cell enlargement or long-distance transport of anions (by playing the role of accompanying cation), it has been qualified as a "functional" nutrient in these species [39, 161]. Furthermore, in some plant species, partial replacement of K^+ by Na^+ can have beneficial effects even under adequate K^+ supply. In sugar beet, when 2.5 mM K^+ + 2.5 mM Na^+ replaced 5 mM K^+ in the nutrient solution, an increase in plant dry weight and sucrose concentration in the storage root was observed [38]. Thus, while most of the research programs on Na^+ in plants have been oriented towards the investigation of plant adaptation to salinity, it is clear that considering Na^+ only as a toxic ion whose uptake, translocation, and accumulation have to be tightly controlled by the plant to prevent stressing effects would be a simplistic analysis.

In halophytes, the effect of Na^+ on growth varies amongst species. Many, but not all, dicotyledonous halophytes need moderate NaCl concentrations (100–200 mM NaCl) to show optimal growth, while many monocotyledonous halophytes can display normal growth in the absence of salt or are stimulated by low NaCl concentrations, in the mM range [62, 63].

4.5.3 Essential Roles of Na^+ in Some Plant Species

Na^+ is an essential element in some plants displaying C_4 photosynthetic activity, like *Atriplex vesicaria* [162], *Echinochloa utilis* (Japanese millet), and *Portulaca grandiflotra* (rose moss) [163]. Low external concentrations of Na^+ (ca. 0.1 mM) are needed by such plants to avoid chlorosis, necrosis, and failure to set flowers even in presence of high external K^+ concentrations (>5 mM) [163]. This requirement for Na^+ is thought to reflect the involvement of this cation in at least two processes [10].

First, Na^+ has been shown to facilitate the conversion of pyruvate into phosphoenolpyruvate (PEP) (an important substrate in carbon fixation in C_4 plants), which occurs in leaf mesophyll cells prior to the Calvin cycle [164]. The molecular mechanisms underlying the effect of Na^+ in this process are however still poorly understood.

Second, it facilitates the translocation of pyruvate (which is central to the CO_2-concentrating mechanism in C_4 species) into chloroplasts [165, 166]. A transport system localized at the chloroplast envelope membrane and endowed with

Na^+-dependent pyruvate transport activity has been identified at the molecular level [24]. The identified gene (*BASS2*) is present in all plants but the encoded protein is especially abundant in plants of the sodium-dependent C_4 type [24, 25]. To our knowledge, the BASS2 protein is the only transport system identified in higher plants so far as displaying Na^+ co-transport activity.

5 Channels and Transporters Involved in Potassium and Sodium Transport in Plants

Molecular and functional analyses indicate that at least 3 families of channels, named Shaker, TPK/KCO and TPC, and 3 families of transporters, named HAK, HKT and CPA, contribute to K^+ and/or Na^+ membrane transport (uptake, distribution and compartmentalization) in plants (Figure 2).

In total, these families gather at least 70 members in the model plant *Arabidopsis thaliana*. The dichotomic classification of membrane transport systems into channels and transporters is based on the mechanisms underlying ion permeation [167, 168]. When open, channels can be regarded as selective pores through which ions move without inducing any change in the general conformation of the protein. In contrast, transporters undergo a cycle of conformational changes for each solute they transport. The maximum velocity of action of channels (up to 10^6 to 10^7 ions per second and protein) can thus be much higher than that of transporters (in the range 10–10^3 transport events per second and protein).

Furthermore, ions move always down their electrochemical gradient (passive transport) in channels, while they can move against their gradient (active transport) in transporters. The latter movement can be coupled to that of another substrate down its own electrochemical gradient, for instance H^+ [168]. It should be noted that the ionic selectivity of the transport protein does not *per se* constitute a criterion for discriminating transporters from channels since each of these categories of proteins comprise members endowed with a high or low ionic selectivity. It should also be noted that rigid dichotomization between channels and transporters is increasingly proving to be too simplistic to describe the functional diversity of these proteins [169–171].

5.1 Families of K^+-Selective Channels Identified at the Molecular Level

The two protein families, named Shaker and TPK/KCO, that have been described in plants as forming K^+-selective channels have counterparts in the animal kingdom: animal Shaker (Kv) channels for the former family [172] and K2P for the latter one [173]. K^+ channels have evolved to fulfill different functions in plants, some of which can be considered as strictly plant specific, as described below.

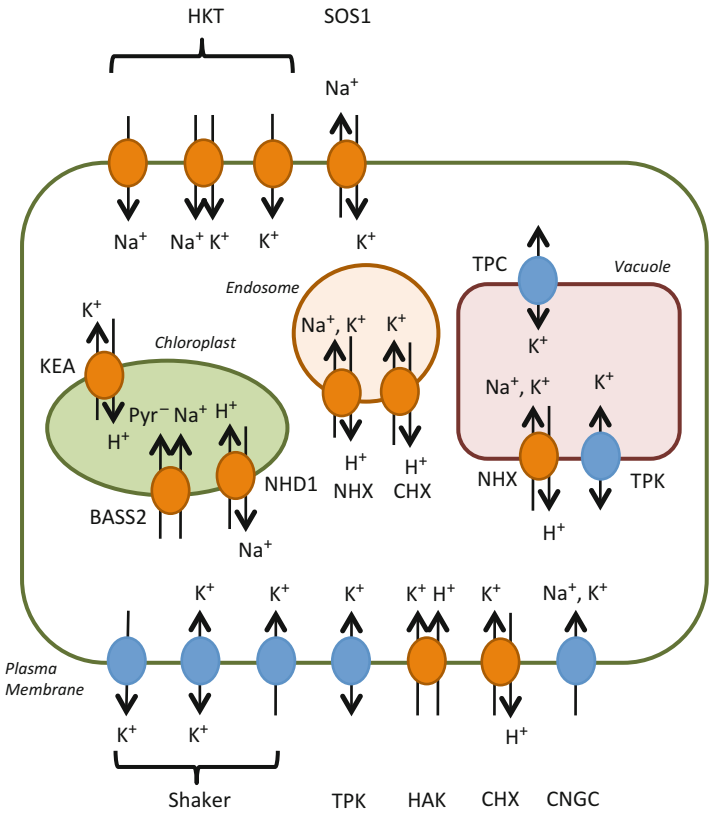

Figure 2 Schematic representation of the current knowledge of subcellular localization of members of the different families of channels and transporters permeable to Na^+ and/or K^+ in plants. Channels and transporters are displayed in blue and orange, respectively. Abbreviations: BASS2 = bile acid/Na^+ symporter family protein 2; CHX, cation/H^+ exchanger; CNGC, cyclic nucleotide-gated channels; HAK, high-affinity K^+ transporter; HKT, high-affinity K^+ transporter; KEA, K^+ efflux antiporter; NHD1, Na^+/H^+ antiporter; NHX, Na^+ and/or K^+-H^+ exchanger; SOS1, salt-overly sensitive 1 protein (NHX family); TPC, two-pore channel; TPK, tandem-pore K^+ channel. Some families are specific of a single type of membrane. This is the case of Shaker channels and HKT transporters, which are active at the plasma membrane, and of the TPC family, which display activity at the vacuolar membrane. Other families contribute to ion transport through different types of membrane. Of course, not all these proteins are expressed in the same cell type.

5.1.1 Shaker Channels

Plant Shaker channels form the main K^+ conductance at the plasma membrane in most cell types in plants (Figure 2). These channels are regulated by voltage like their animal counterparts. Functional channels result from the assembly of four subunits (alpha-subunits) that form a permeation pathway for K^+ in the center of the structure. It is worth to note that the four subunits can be the product of a single

Shaker gene (homomeric channels) or different Shaker genes (heteromeric channels) [172]. A Shaker alpha-subunit consists of a hydrophobic core displaying six transmembrane segments with both N- and C-terminal regions located on the cytosolic side of the membrane.

Two modules can be distinguished in the transmembrane core: a voltage-sensing module comprising the first four transmembrane segments (S1-S4) and a pore-forming module (S5-P-S6). In the former module, the fourth transmembrane segment (S4), which is enriched in positively charged residues, constitutes the voltage sensor. Between the fifth (S5) and sixth (S6) transmembrane segments of the pore-forming module, a pore loop (P) is located, harboring the canonical K^+ selectivity filter "TxGYG" (Thr-X-Gly-Tyr-Gly) like in animal Shaker channels. The cytosolic C-terminal part which begins just after the end of the sixth transmembrane segment (S6), contains the following domains (successively from N- to C-term): a C-linker (about 80 residues in length) [174], a cyclic-nucleotide binding domain (CNBD), an ankyrin domain (absent in some alpha-subunits) [175, 176], and a KHA domain rich in hydrophobic and acidic residues [177].

So far, there are four functional types of plant Shaker channels, which have been well documented in the model plant *Arabidopsis thaliana*. These four functional types fall into five phylogenetic groups in this species [178]. Groups 1 and 2 include five inwardly-rectifying channels: AKT1, SPIK, and AKT6 in group 1, and KAT1 and KAT2 in group 2 (Figure 2). All these channels are voltage-gated and open at hyperpolarized membrane potentials. Group 3 has AKT2 as only member, which behaves as a weakly-rectifying channel. AKT2 weakly-rectifying currents can be decomposed into two components: a voltage-independent ("Ohmic" like) component mediated by channels that are open at all membrane potentials, and an inwardly-rectifying component mediated by channels that only open at hyperpolarized potentials (like channels from groups 1 and 2) [179].

Both components correspond to two channel states and it is expected that changes between these two states are phosphorylation-dependent [180]. Like group 3, the Arabidopsis Shaker group 4 comprises a single member, named AtKC1, which seems unable to form functional channels by itself but is able to interact with alpha-subunits from groups 1, 2, and 3 to form functional heteromeric channels with distinctive functional features. It is thus regarded as a regulatory subunit. The last group, group 5, comprises two outwardly-rectifying channels, named SKOR and GORK. These two channels are voltage-gated (like the channels formed by members from group 1 to 4) but they open at depolarized membrane potentials. Thus, in Arabidopsis, 6 Shaker genes give rise to voltage-gated inwardly rectifying channel activity, 2 genes give rise to voltage-gated outwardly-rectifying channels and one gene to weakly-rectifying channel activity (mediated by homotetrameric AKT2 channels). It is worth to mention that the same basic subunit topology is found among all these channels with the exception of the ankyrin domain, which is absent in groups 1, 2, and 4.

Arabidopsis has also served as a model for determining the physiological role of many of the aforementioned Shaker functional types. Heteromeric AKT1/AtKC1 and/or homomeric AKT1 channels contribute to a large extent to K^+ uptake in root

cells [102, 181–183]. Due to the hyperpolarized membrane potentials recorded in root cells, AKT1 channels can take up K^+ from solutions containing K^+ concentrations as low as 10 μM [102, 184, 185]. KAT1 and KAT2 are strongly expressed in guard cells where they mediate K^+ uptake, which leads to guard cell swelling and stomatal opening [186]. GORK is expressed in guard cells and in root periphery cells where it mediates K^+ efflux. In guard cells, this efflux gives rise to decreased turgor and stomatal closure [108]. In root periphery cells and root hairs, it could play a role in osmoregulation and possibly in signal transduction [187]. SKOR and AKT2 are preferentially expressed in vascular tissues where they take part in long-distance K^+ transport.

SKOR is expressed in root stele cells (pericycle and xylem parenchyma cells) where it mediates K^+ secretion into the xylem sap [78]. AKT2 is mainly expressed in the phloem where it contributes to K^+ load and/or unload in source and sink tissues [188, 189]. It is noteworthy that AKT2-mediated control of membrane potential (by modulating K^+ fluxes) can constitute an energy source for sucrose loading into the phloem [190]. Besides, AKT2, together with AKT1, contributes to K^+ uptake in mesophyll cells [191]. Finally, AKT6 and SPIK are expressed in reproductive tissues [189]. SPIK channels form a large K^+ inward conductance in pollen that permits germination and turgor-dependent growth of pollen tubes [192].

5.1.2 TPK/KCO Channels

The tandem-pore K^+ (TPK) channel family comprises 6 members (TPK1-TPK5 and KCO3) in the model plant *Arabidopsis thaliana*. TPK channels fall into two phylogenetic groups: TPK1 on one side and TPK2, TPK3, TPK4, and TPK5 on the other [193]. Each subunit consists of four transmembrane segments (TM) and two pore loops (P), containing the canonical K^+ selectivity filter "TxGYG", that are arranged as TM-P-TM-TM-P-TM. KCO3 is a special case since it only possesses two transmembrane segments that are separated by a pore domain (TM-P-TM topology), a structure reminiscent of that of animal Kir and bacterial KcsA channels [194–196]. The encoding *KCO3* gene is thought to have originated by gene duplication of the *TPK2* gene, followed by a partial deletion that resulted in the loss of one pore domain (a TM-P-TM module) [173, 197].

It is worth to note that plant TM-P-TM subunits have been only found in the Arabidopsis genus so far [193]. Like Shaker alpha-subunits, TPKs subunits have both their N- and C-terminal ends located in the cytosol. Binding sites for 14-3-3 proteins are found in the N-terminus while one or two Ca^{2+}-binding EF hands are present in the C-terminus. Functional TPK channels arise from the assembly of two subunits that leads to the formation of a central pore with four pore domains. Unlike plant Shaker channels, TPK channels do not have a voltage sensor domain and are thus not regulated by voltage [197]. Instead, they are regulated by Ca^{2+}, via the EF hands, and by pH. Expression analyses of *TPKs* genes have shown that the highest transcript levels in root, leaves and flowers is displayed by *TPK1*, followed by *TPK3* and *TPK5* [173]. Expression of *TPK2* and *TPK3* was essentially detected in flowers,

but with low transcript levels. *TPK4* expression seems to be restricted to pollen as observed by *promoter-GUS* (β-glucuronidase) fusion analysis [198]. Albeit some TPK subunits are co-expressed in the same cell types, TPK channels seem to only result from homodimer assembly [199].

At the subcellular level, TPK1, TPK2, TPK3 and TPK5 are localized to the tonoplast (Figure 2) as shown by fusions to the fluorescent marker GFP [199]. In contrast, TPK4 seemed to be partly targeted to the plasma membrane, while an important fraction remained in the ER [198, 200]. Although general targeting sequences have not been identified in Arabidopsis TPKs, localization of rice TPKs to lytic vacuole tonoplast or to protein storage vacuoles relied on a reduced number of residues located in the C-terminus of the channel subunits [201]. Information about the physiological role of TPK channels in plants is limited at the present time. They are expected to participate in intracellular K^+ transport that involves vacuoles and organelles. For instance, TPK1 participates in vacuolar K^+ release necessary for stomatal closure and seed germination [202]. Interestingly, several vacuolar TPKs, including AtTPK1, seem to respond to mechanical stimulation, suggesting a link between osmoregulation and TPK-mediated K^+ transport [203].

5.2 K^+-Permeable Transporters from the HAK/KUP/KT Family

Plant HAK/KUP/KT transporters were first identified from their homology to bacterial KUP (K^+ Uptake) and fungal HAK (high-affinity K^+) transporters [204, 205]. The first plant transporters identified in this family were cloned from barley (HAK1 [206]) and Arabidopsis (KUP1/KT1, KUP2/KT2; K^+ transporter [207–209]). Due to the different acronyms used in these early reports, the composite name of HAK/KUP/KT is widely used to refer to the whole family in plants. Functional characterization in yeast and/or bacteria mutants devoid of endogenous K^+ uptake systems has clearly evidenced permeability to K^+ in various members of this family. Data obtained from Arabidopsis confirm that some members of the family do indeed play a role in K^+ homeostasis in roots [185, 210–212] and shoots [213]. The HAK/KUP/KT transporters characterized so far in plants are mainly permeable to K^+. Their capacity to discriminate between this cation, Rb^+ and Cs^+ is however low [172], while it is high between K^+ and Na^+, with a 10^3 difference in their corresponding apparent affinity constant (K_M close to 10 μM for K^+ and 10 mM for Na^+) [206, 214].

Based on hydropathy profiles, HAK/KUP/KT transporters would possess from 10 to 14 transmembrane segments [210]. In contrast to the Shaker and TPK families, the HAK/KUP/KT one displays a high and rather variable number of members in the different plant species genomes that have been sequenced so far. For instance, the genome of the dicot model Arabidopsis and that of the monocot model rice displays 13 and 27 genes, respectively. The physiological significance of such differences is still poorly understood [172].

HAK/KUP/KT transporters are generally classified according to their sequence homology into four clusters (I–IV [215]). Cluster I comprises well characterized

transporters like AtHAK5 or OsHAK1, which have been shown to mediate K^+ uptake in roots from low external concentrations, probably by mediating H^+:K^+ co-transport (Figure 2) [214, 215]. Such capacity allows plants to thrive under low-K^+ conditions [211, 212]. So far, characterization of members belonging to cluster II has revealed a striking diversity in terms of transport properties and physiological roles. In Arabidopsis, they seem to be involved in developmental processes dependent on, or resulting in, cell expansion [213, 216, 217]. Little information is available on cluster III and cluster IV transporters.

5.3 Na^+-Selective Transporters

5.3.1 High-Affinity K^+ Transporters

High-affinity K^+ transporters (HKT) transporters are related to fungal and bacterial K^+ transporters from the Trk/Ktr families [194]. In plants however, HKT transporters display varying Na^+/K^+ permeabilities. When characterized in heterologous systems, 3 main types can be distinguished: K^+- or Na^+-selective transporters and Na^+-K^+ symporters (Figure 2) [218–221]. Initial sequence analyses and *in silico* modelling suggested that HKT transporters contain a hydrophobic core with four "MPM" domains (a MPM domain being formed by a pore loop surrounded by one transmembrane domain at each side), the N- and C-terminus being located in the cytosol. The four MPM domains assemble in such a way that the four pore loops are located at the center of the hydrophobic structure to form part of the permeation pathway. Recent crystallization of bacterial Trk/Ktr homologues has provided strong support to this configuration [222, 223].

Phylogenetic and functional analyses have led to sort HKT transporters into 2 subfamilies [224]: subfamily I, present in both monocotyledonous and dicotyledonous species, and subfamily II, identified only in monocotyledonous species so far [224]. In Arabidopsis, a single *HKT* gene, *AtHKT1,* has been identified [221] while in rice, eight or nine *HKT* genes exist depending on cultivars [225, 226]. Subfamily II HKT transporters are expected to be all K^+-permeable and can operate as Na^+-K^+ symporters [218, 226] or K^+-selective uniporters [220, 227] when heterologously expressed in yeast and/or *Xenopus* oocytes. Transporter permeability to K^+ relies on a conserved glycine residue in the middle of the selectivity filter of HKT/Trk/Ktr transporters [222, 223, 228]. In subfamily I HKT transporters, such a glycine is absent [229]. Subfamily I HKT transporters are Na^+-selective in Arabidopsis and rice [50, 53, 218, 221]. In other species, they are expected to be Na^+-selective as well because of the absence of conserved glycine in their selectivity filter [224], although indication of K^+-permeable HKT transporters within the dicotyledonous subfamily I has been reported [230–232].

Concerning their physiological role, HKT transporters have been widely associated to Na^+ transport and tolerance to Na^+ stress [172]. The AtHKT1 transporter from Arabidopsis strongly contributes to Na^+ recirculation from shoots to roots,

contributing to reduce the plant sensitivity to Na^+ [50, 233]. AtHKT1 is expressed in root xylem parenchyma and root and shoot phloem and it prevents shoot Na^+ over-accumulation both by limiting the amount of Na^+ delivered to the shoots, through xylem sap desalinization, and by recirculating shoot Na^+ to the roots via the phloem sap [50, 234]. Genetic analyses have shown that several subfamily I HKT transporters, in both dicotyledonous and monocotyledonous species, are associated to QTLs of salt tolerance by limiting leaf Na^+ accumulation upon salt stress [51, 53, 235]. Less information about the role of subfamily II HKT transporters has been reported. In rice, OsHKT2;1 provides a major pathway for root high-affinity Na^+ uptake that supports plant growth under limiting K^+ supply [157]. Its possible involvement in root K^+ uptake has not been evidenced yet.

5.3.2 Na^+:Pyruvate Cotransporters

Recently, a plastidial protein named BASS2 (for "bile acid sodium symporter family" protein 2) has been characterized in several C_3 and C_4 species, including *A. thaliana,* and shown to mediate pyruvate:Na^+ co-transport into chloroplasts (Figure 2) [24]. Orthologues of BASS2 can be detected in all the land plant genomes that have been sequenced so far. Interestingly, such pyruvate:Na^+ co-transport mechanism was well established in C_4 species but not well characterized in the C_3 ones [25, 236]. In C_3 species, BASS2 function would supply pyruvate to the MEP pathway [24, 237]. To mediate pyruvate:Na^+ co-transport, it has been proposed that BASS2 requires a Na^+ gradient established by NHD1 Na^+/H^+ antiport activity (member from the NhaD family; see below).

5.4 Monovalent Cation/H^+ Antiporters from the CPA Superfamily

This superfamily of cation/H^+ antiporters (CPA) comprises three major families designated as CPA1, CPA2, and NhaD [238–240]. In plants, CPA1 includes the well-studied Na^+-K^+/H^+ exchanger (NHX) family, CPA2 includes the K^+ efflux antiporter (KEA) and cation-H^+ exchanger (CHX) families and NhaD includes Na^+/H^+ antiporters.

CPA1 transporters, which are predicted to have 10 to 12 transmembrane segments, mediate electroneutral Cation/H^+ exchange [241]. CPA1-type transporters are found in all kingdoms, including archaea, bacteria, fungi, plants and metazoa [238]. In plants, CPA1 transporters behave as Na^+/H^+ and/or K^+/H^+ antiporters (Figure 2). They are involved in salt tolerance and K^+ homeostasis by contributing either to cation compartmentalization in cellular organelles, as shown for instance for NHX transporters, or to Na^+ extrusion from the cell, as shown for the SOS1 Na^+:H^+ antiporters [242]. Arabidopsis contains eight isoforms belonging to three

classes: two divergent members localized to the plasma membrane (SOS1/AtNHX7 and AtNHX8), and six intracellular isoforms that are targeted to the vacuolar (AtNHX1 to AtNHX4) or endosomal membranes (AtNHX5, AtNHX6). AtNHX1 and AtNHX2 contribute to vacuolar K^+ accumulation and pH homeostasis that is required for plant growth, stomatal functioning and flower development [125, 243, 244]. AtNHX5 and AtNHX6 seem to be involved in the establishment of pH gradients between organelles that are important for vacuolar trafficking. Interestingly, cell expansion and plant growth is greatly reduced in plants lacking both AtNHX5 and AtNHX6, which highlights the relevance of endosomal pH and ion homeostasis in these physiological processes [245, 246]. Concerning salt tolerance, these two types of NHX transporters, vacuolar or endosomal, display contrasting responses to sodium excess. Disruption of *AtNHX5* and *AtNHX6* renders plants salt-sensitive. In contrast, moderate salt can complement growth and flower defects displayed by mutants lacking AtNHX1 and AtNHX2 [244, 245]. A consistent observation is that improved Na^+ compartmentalization into the vacuole by overexpression of vacuolar NHX proteins results in increased tolerance of the plant to a salinity constraint [242]. In addition to the contribution of NHX transporters to vacuolar Na^+ accumulation, selective Na^+ excretion from the cell by plasma membrane SOS1-like transporters has been widely shown to limit Na^+ levels in the cytosol and thereby to improve plant performance under salt stress [76]. Furthermore, SOS1 activity appears to protect root plasma membrane K^+ uptake capacity from external media displaying high Na^+ concentrations [247].

Six *KEA* genes (AtKEA1 to 6) are present in the Arabidopsis genome. AtKEA1 and AtKEA2 are targeted to the chloroplast inner envelope membrane whereas AtKEA3 is targeted to the thylakoid membrane. By using Arabidopsis plants mutated in AtKEA1 to 3 transporters (single, double, and triple mutants), it has been shown that these transporters play an essential role in chloroplast osmoregulation, integrity, and ion and pH homeostasis (Figure 2) [248].

The Arabidopsis CHX family, with twenty-eight members in *A. thaliana*, is much larger than the KEA one. It comprises transporters targeted to the plasma membrane, prevacuolar membrane or the endoplasmic reticulum, where they exchange K^+ against H^+ [249–251]. Association of AtCHX proteins with endomembranes and their roles in pH and cation homeostasis suggest that these proteins play important roles in membrane trafficking, similarly to endosomal NHX transporters. AtCHX20 antiport activity in endosomes contributes to guard cell swelling and stomatal opening [126]. In Arabidopsis pollen, AtCHX21 and AtCHX23 are involved in either the reception or the transduction of female signals that target pollen tube to the ovule [250]. Intriguingly, multiple CHX genes are expressed in Arabidopsis pollen, but this is not well understood yet.

NhaD-type carriers have been identified in all vascular and non-vascular plants, including mosses and algae [252]. In *A. thaliana*, the Na^+/H^+ antiporter AtNHD1 has been shown to participate in Na^+ export from chloroplasts, a process that contributes to salt tolerance, efficient photosynthesis and plant performance (Figure 2) [253]. AtNHD1 may work in parallel to KEA exchangers at the chloroplast envelope with overlapping substrate specificity [248]. Evidence has also been obtained

that NHD1 energize BASS2 pyruvate/Na^+ co-transport into the chloroplast (see Section 5.3.2) by establishing an inwardly-directed sodium gradient across the envelope [24].

5.5 Poorly Selective Transport Systems Permeable to K^+ and Na^+

5.5.1 Cyclic Nucleotide-Gated Channels

Cyclic nucleotide-gated channels (CNGCs) share structural homology with Shaker channels as they have six transmembrane segments and a long cytosolic C-terminal domain harboring a CNBD. In contrast, they lack the canonical motif TxGYG, hallmark of K^+-selective channels [66, 254]. Unlike Shaker channels, they seem to be regulated by cGMP and/or cAMP and to poorly discriminate among monovalent cations (Figure 2) [255, 256]. A notable exception is AtCNGC2, which exhibited a high degree of K^+ selectivity as opposed to Na^+ [257], a feature that is unknown in animal CNGCs [258].

Despite the fact that CNGCs seem to play a prominent role in plant immunity by probably mediating Ca^{+2} fluxes [259, 260], some of them have shown features related to K^+ and/or Na^+ transport. For instance, AtCNGC10 was shown to rescue K^+ transport mutant strains of *Escherichia coli* (LB650) and yeast (CY162), and the Arabidopsis *akt1* mutant [261]. In heterologous systems, AtCNGC3 can mediate Na^+ and K^+ uptake [129]. Moreover, promoter-driven GUS activity data has shown that AtCNGC3 is mainly expressed in epidermal and cortical root tissues in seedlings, a feature consistent with a role in K^+ and/or Na^+ transport.

5.5.2 Tandem-Pore Channels

Tandem-pore channels (TPC) proteins share structural similarities with voltage-gated cation channels, since one polypeptide consists of two repeats of the basic S1-S6 structure present in Shaker K^+ channel. Unlike their animal homologues, plant TPC channels are targeted to the tonoplast and are responsible for the so-called slow vacuolar (SV) currents (Figure 2) [262, 263]. They are activated by a rise in cytosolic calcium concentration and do not discriminate among cations, either monovalent or divalent [264–269]. In the presence of low Na^+ concentrations and of a K^+ gradient directed into the cytosol, a TPC channel is able to transport K^+ across the tonoplast in either direction, depending on the electrochemical driving forces [262]. In Arabidopsis, phenotype analyses of plants displaying a loss-of-function mutation in the single TPC family member has revealed that this channel contributes to seed germination and stomatal movements [263], but the physiological significance of these observations remains unclear.

6 General Conclusions

Important concerns and objectives, both at the biological and agricultural levels, are underlying research on roles and transport of K^+ and Na^+ in plants. K^+ is an essential element, which is required in large quantities while its availability in the soil solution is often low, in the μM range, and limiting for optimal plant growth. It is involved in a large number of crucial functions, among which are osmocontractility, for instance in guard cells which regulate the aperture of stomatal pores and gas exchanges at the leaf surface. In contrast, Na^+ is not an essential element in most plants. The major issue regarding this ion is that its concentration in the soil can be high and thus toxic, preventing plant growth and agriculture in a large proportion of the arable lands.

In the context of such concerns, efforts have been particularly made to identify and characterize channels or transporters involved in K^+ or Na^+ transport. Major advances have been made during the last 2 decades in this domain, using DNA-based strategies, cell biology, (electro)physiology, genetics and reverse genetics, and whole plant biology. For instance, this has provided valuable knowledge on transport mechanisms responsible for K^+ uptake from the soil solution [75], K^+ fluxes in guard cells [121], and Na^+ exclusion [73] or compartmentalization into vacuoles [91].

However, we are very far from having a holistic view of the functional properties and roles of the tens of Na^+ and K^+ transport systems that have been identified in the genome of the model plant *Arabidopsis thaliana* [270], and still farther from understanding the physiological significance and consequences of the differences that are revealed by phylogenetic comparison of the different families of transport systems between plant species [172]. Further progress in this direction is clearly required. It seems very reasonable to expect that this will provide new tools and strategies to improve, for instance, plant K^+ use efficiency or tolerance to soil salinity.

Abbreviations

ADP	adenosine diphosphate
AKT	Arabidopsis K^+ transport system
AtKC1	*Arabidopsis thaliana* K^+ channel 1
ATP	adenosine 5′-triphosphate
BASS2	bile acid:Na^+ symporter family protein 2
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanidine monophosphate
CHX	cation/H^+ exchanger
CNBD	cyclic nucleotide binding domain
CPA	cation/H^+ antiporter
ER	endoplasmatic reticulum

GFP	green fluorescent protein
GORK	gated outwardly-rectifying K^+ channel
GS/GOGAT	glutamine synthetase/glutamine oxoglutarate aminotransferase
GUS	β-glucuronidase
HAK	high-affinity K^+ transporter
KAT	K^+ channel in *Arabidopsis thaliana*
KEA	K^+ efflux antiporter
KT	K^+ transporter
KUP	K^+ uptake transporter
MEP	methyl erythritol phosphate
NHX	Na^+-K^+/H^+ exchanger
P	pore loop
PEP	phosphoenolpyruvate
PEPC	phosphoenolpyruvate carboxylase
PK	pyruvate kinase
QTL	quantitative trait locus
SKOR	stellar K^+ outward rectifier
SPIK	Shaker pollen inward K^+ channel
TM	transmembrane segment

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