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Review

Adaptation of Plants to Salt Stress: Characterization of Na^+ and K^+ Transporters and Role of CBL Gene Family in Regulating Salt Stress Response

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Received: 14 September 2019; Accepted: 21 October 2019; Published: 28 October 2019

Abstract: Salinity is one of the most serious factors limiting the productivity of agricultural crops, with adverse effects on germination, plant vigor, and crop yield. This salinity may be natural or induced by agricultural activities such as irrigation or the use of certain types of fertilizer. The most detrimental effect of salinity stress is the accumulation of Na^+ and Cl^- ions in tissues of plants exposed to soils with high NaCl concentrations. The entry of both Na^+ and Cl^- into the cells causes severe ion imbalance, and excess uptake might cause significant physiological disorder(s). High Na^+ concentration inhibits the uptake of K^+, which is an element for plant growth and development that results in lower productivity and may even lead to death. The genetic analyses revealed K^+ and Na^+ transport systems such as SOS1, which belong to the CBL gene family and play a key role in the transport of Na^+ from the roots to the aerial parts in the *Arabidopsis* plant. In this review, we mainly discuss the roles of alkaline cations K^+ and Na^+, Ion homeostasis-transport determinants, and their regulation. Moreover, we tried to give a synthetic overview of soil salinity, its effects on plants, and tolerance mechanisms to withstand stress.

Keywords: salinity; sodium; potassium; ion homeostasis-transport determinants; CBL gene family

1. Introduction

The adverse effects of salinity on plant growth are generally associated with the low osmotic potential of the soil solution and the high level of toxicity of sodium (and chlorine for some species) that causes multiple disturbances to metabolism, growth, and plant development at the molecular, biochemical, and physiological levels [1,2]. In vitro experiments have shown that the enzymes extracted from the halophyte plants *Triplex spongeosa* or *Suaeda maritima (L.)* are sensitive to NaCl to the same degree as those extracted from the glycophyte plants [3,4]. These experiments suggest that tolerance to salinity is not limited to a metabolic response in tolerant plants. Generally, sodium begins to have an inhibitory effect on enzymatic activity from a concentration of 100 mmol/L. Thus, the ability of plants to reduce sodium levels in the cytoplasm appears to be one of the decisive factors in salinity tolerance [5,6]. However, although chloride ions are micro-elements necessary as co-factors, for enzymatic activity, photosynthesis, and the regulation of cell turgor, pH, and electrical membrane potential, they remain no less toxic than Na^+ ions if their concentration reaches the critical threshold tolerated by plants [7]. Ionic cellular homeostasis is an essential and vital phenomenon for all organisms. Most cells maintain a high level of potassium and a low level of sodium in the cytoplasm through the coordination and

regulation of different transporters and channels. There are two main strategies that plants use to cope with salinity—The compartmentalization of toxic ions within the vacuole and their exclusion outside the cell [5,6]. On the other hand, plants modify the composition of their sap; they can accumulate Na^+ and Cl^- ions to adjust the water potential of tissues necessary to maintain growth [6]. This accumulation should be consistent with a metabolic tolerance of the resulting concentration or with compartmentalization between the various components of the cell or plant. It requires relatively little energy expenditure. If this accumulation does not take place, the plant synthesizes organic solutes to adjust its water potential. It will require a large amount of biomass to ensure the energy expenditure necessary for such a synthesis. Therefore, one adaptation strategy consists of synthesizing osmoprotective agents, mainly amino compounds and sugars, and accumulating them in the cytoplasm and organelles [8,9]. These osmolytes, usually of a hydrophilic nature, are slightly charged but polar and highly soluble molecules [10], suggesting that they can adhere to the surface of proteins and membranes to protect them from dehydration. Another function attributed to these osmolytes is protection against the action of oxygen radicals following salt stress [11]. Under high sodium concentration levels, whether the latter is compartmentalized within the vacuole or excluded from the cell, the osmotic potential of the cytoplasm must be balanced with that of the vacuole and the external environment in order to maintain the cell turgor and the water absorption necessary for cell growth. This requires an increase in osmolyte levels in the cytoplasm, either by the synthesis of solutes (compatible with cellular metabolism) or by their uptake of the soil solution [12,13]. Among these synthesized compounds are some polyols, sugars, amino acids, and betaines, which, energetically, are very expensive to be produced by the cell [14]. The main role of these solutes is to maintain a low water potential inside the cells to generate a suction force for water absorption. Furthermore, the involvement of solutes such as glycine betaine, sorbitol, mannitol, trehalose, and proline in improving tolerance to abiotic stress has been demonstrated by genetic engineering and plant transgenesis [6,14,15]. On the other hand, salt stress induces the production of active forms of oxygen following the alteration of metabolism in the mitochondria and chloroplasts. These active forms of oxygen cause oxidative stress whose adverse effects are reflected in various cellular components such as membrane lipids, proteins, and nucleic acids [16]. As a result, the reduction of these oxidative damages through the deployment of a range of antioxidants could contribute to improving plant tolerance to stress [17]. Early events in plant stress adaptation begin with mechanisms of perception and signaling via signal and messenger transduction to activate various physiological and metabolic responses, including the expression of stress response genes. The main pathways activated during the salt stress signaling include calcium, abscisic acid (ABA), mitogen-activated protein kinases (MAPKinases), salt overly sensitive proteins (SOS), and ethylene [12]. In this chapter, we mainly discuss roles of alkaline cations K^+ and Na^+, ion homeostasis-transport determinants, and their regulation. Furthermore, we tried to give a hypothetical overview of soil salinity, its effects on plants, and tolerance mechanisms that allow the plants to withstand stress. A fundamental biological understanding and knowledge of the effects of salt stress on plants is needed to provide additional information for the study of the plant response to salinity and try to find other way for improving the impact of salinity in plants and accordingly enhance crop yields to cope with the starvation that persists in some parts of the world

2. Roles of Alkaline Cations K^+ and Na^+ in Plants

Potassium (K) is the third of the three primary nutrients required by plants, along with nitrogen (N) and phosphorus (P). Potassium, with about 100 to 200 mM concentration in the cytosol, is the major inorganic cation of the cytoplasm in plant and animal cells. The reasons for its preferential accumulation compared to Na^+ is probably due to the fact that Na^+ is more "chaotropic" (because of its smaller size and stronger electric field on its surface) [18].

Na^+ is not an essential nutrient for higher plants. For a high concentration of Na^+ in the soil, this cation becomes even toxic to the plant. At lower concentrations, the plant can use it beneficially as a vacuolar osmoticum.

2.1. Physiological Roles

As most inorganic cations are abundant in the cytoplasm, the potassium is involved in critical cell functions. In addition to its role in the neutralization of the net electric charge of biomolecules, the potassium participates, for example, in membrane transport processes, enzyme activation, and osmotic potential. In plants, in conjunction with osmotic potential [19], K^+ is involved in the control of the turgor pressure [20] and related functions, cell elongation and cell movement. Finally, K^+ plays a direct or indirect role, in the regulation of enzyme activities, the protein synthesis, photosynthesis and homeostasis of the cytoplasmic pH.

These different roles at the cellular level involve potassium in essential functions at the level of the whole plant, for example gas exchange control via regulation of the opening and closing of the stoma, the xylem sap ascension by root thrust, installation of potential osmotic gradient carrying phloem sap flow from original organs to hole organs or even port of herbaceous species.

2.2. Effect of K^+ Deficiency on Plants Physiology

In K^+ deficiency, the sap flow is disturbed, with spontaneous reduction of the phloem sap velocity of circulation. The photoassimilates then accumulate inside of the leaves. Symptoms of chlorosis and necrosis from the photooxidation of the photosynthetic system are frequently observed. It is well settled that K^+ deficiency induces the acidification of the extracellular medium. Minjian et al. [21], showed that root K^+ absorption depends on the activity of the proton pumps (H^+-ATPases) and the occurrence of K^+ transporters on the cellular membrane. The level of H^+ expulsion can be used as a criterion of tolerance to K^+ deficiency. Chen and Gabelman [22] observed in tomato strains that K^+ uptake efficiency is associated with a high net K^+ influx coupled with low pH around root surfaces. The proton-electrochemical gradient may contribute to energizing K^+ uptake, and indeed it is used by some KT/ KUP/HAK transporters, which co-transport K^+ and H^+ [23].

2.3. Toxicity of Na^+ in the Cytoplasm

In plants, the concentration of Na^+ in the cytosol is maintained at a lower value than that of K^+ in animals. In animal cells, the concentration of Na^+ is closely regulated to 10^{-2}mol L^{-1} value [24]. In plant cells, the concentration of Na^+ does not seem to be subjected to narrow homeostasis. When the plant grows in salinity conditions, the accumulation of Na^+ in the cytoplasm beyond a certain threshold becomes toxic, but this threshold is not clearly determined.

The toxicity of Na^+ in the cytosol would result from its "chaotropic" character by comparison with K^+ [18]. The toxicity of Na^+ would also probably mean its ability to compete with K^+ during the process of fixing important proteins. More than 50 enzymes require K^+ to be active, and Na^+ would not provide the same function [25]. Therefore, a high concentration of Na^+ in the cytoplasm inhibits the activity of many enzymes and proteins, leading to cell dysfunctions. In addition, protein synthesis requires a high concentration of K^+ for tRNA attachment to ribosomes [26], so the translation would also be affected.

2.4. Na^+ Acts as Osmoticum

If the plant cell cannot substitute Na^+ to K^+ in its cytosol, it can do it so in the vacuoles and use Na^+ as osmoticum. Different studies have actually shown that moderate amounts of Na^+ can improve the growth of many plant species [27]. For example, a beneficial "nutritious" effect of Na^+ has been described in tomato [28,29].

It is likely that the beneficial effect of Na^+ can especially be observed in conditions of K^+ deficiency. In these circumstances, a controlled build-up of Na^+ probably helps to ensure the regulation of cell turgor pressure [30,31]. Similarly, a moderate absorption of Na^+ can be beneficial if it helps the plant, for example, to quickly adjust their osmotic potential from the beginning of salt stress.

Despite these physiological observations, the genetic determinants of improving the growth of plants by sodium and genes may be involved in these processes, however, they are still poorly characterized. Research on rice [32] concerning the function in planta of a transporter HKT family provided genetic proof on the fact that an accumulation of Na^+ in K^+ deficiency conditions can promote the growth of the plant.

3. Interaction between K^+ and Na^+ Transport and Adaptation to Salt Stress

The adaptation of the plant to the presence of salt in the soil and salt stress involves various processes, occurring at different levels, from the cell to the whole organism, such as a modification of the metabolic activity leading to the accumulation of organic osmolytes [33], or morphological and developmental changes of the leaves [34]. Within this very complex network of responses, the control of membrane transport activities occurring through a variety of mechanisms, a selective accumulation of K^+ and an exclusion of Na^+ [25,35], appear as a central process. Thus, in a large number of models, from isolated cell culture to the whole plant, adaptation to salt stress appears to be correlated with the ability to selectively remove K^+, to control the Na^+ entrance, and maintain the K^+/Na^+ ratio of the internal contents of these two cations at a high level. In this context, the molecular and functional characterization of membrane transport systems of K^+ and Na^+ is, therefore, a priority objective. It is probable that the capacity of the channels and transporters to discriminate K^+ from Na^+ is essentially based on the difference of intensity of the electric field at the surface of these two ions, which results from their difference in size and hydration energies. The crystallographic resolution of the bacterial potassium channel structure [36,37] provides an example for understanding how carbonyl groups of the polypeptide chain can be spatially distributed along the permeation pathway to substitute, without energy barrier to the hydration shell of the ion.

4. Physiology of K^+ and Na^+ Transport in Plants

4.1. Structure–Function Relationship of the Root

The movement of the mineral elements by the roots and their transfer to the aerial parts involves at least two membrane steps—Ions *sensu stricto* absorption from the soil solution by the epidermal cells, cortical, and contingently endodermic, and the secretion inside the vessels at the level of xylem parenchyma cells. The ions radial movement from the orbital cells of the root to the stela can, in theory, take three paths [38]—The apoplastic pathway (through cell wall), the symplastic pathway (through cytoplasm), or a mixed path passing the ions alternately from apoplastic compartment to the symplastic compartment (Figure 1). Above the cell differentiation zone, the apoplastic path is interrupted by the endoderm of the root. The walls of these cells are impregnated with lignin and suberin. This deposition of hydrophobic compounds forms the framework of Caspary and constitutes a barrier that blocks water and solutes movement. The very close association of the endodermal cell membrane with the Caspary framework forces the ions and water to undergo membrane control to pass the endodermal barrier and migrate into the stele. However, at several levels in the root, the ions can take a direct apoplastic path from the external environment to the xylem: at the apex, where the endodermis is not yet suberized, at the level of endoderm discontinuity, induced by the appearance of the secondary roots [39], and in some species, at the level of some non-suberized endodermal cells, called passage cells which are thought to serve as cellular gatekeepers, controlling access to the root interior [40].

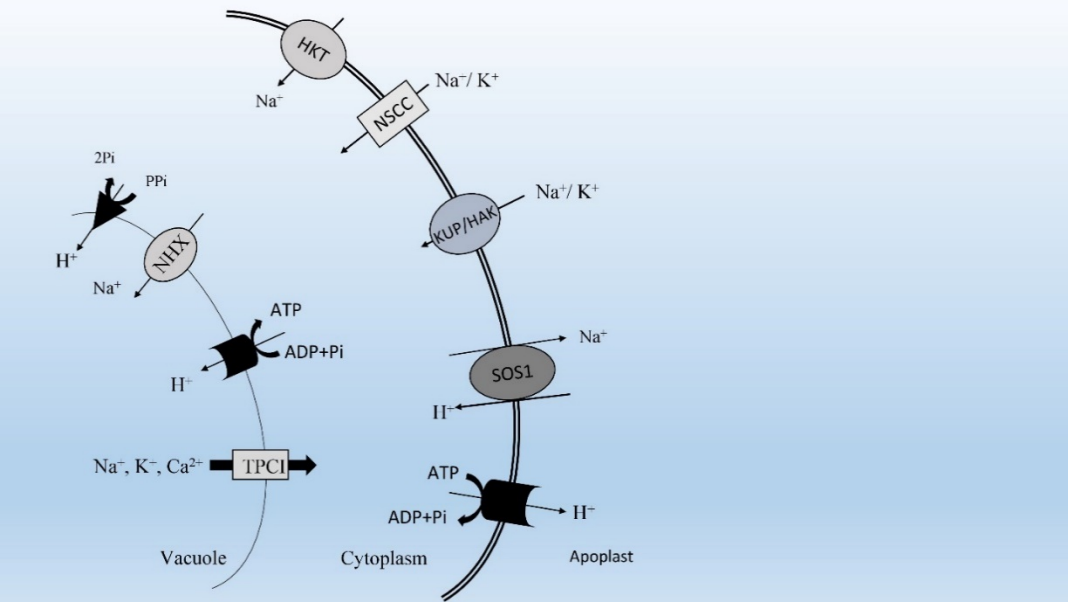

Figure 1. Sodium transport at the cellular level. Schematic representation of transport systems involved in Na^+ transport at the plants through the plasma membrane or the tonoplast. Primary transport systems consisting of proton pump ATPases on the plasmalemma and the tonoplast and a pyrophosphatase on the tonoplast create a pH gradient and a potential difference electric on both sides of the membranes (cytosolic side more alkaline and charged more negatively). Proton concentration gradients allow Na^+ excretion of cytoplasm towards the outside environment or the vacuole via the operation of antiports Na^+/H^+ (appealed SOS1 (Salt Overly Sensitive protein 1) on the plasmalemma or NHX1 (K^+, Na^+/H^+ antiporter), on the vacuole). Potential gradients electric created by the pumps cause the entry of Na^+ in the cytoplasm of the cell since the external environment or the vacuole via non-selective cationic channels (NSCC) (CNGC (Cyclic Nucleotide Gated Channels) on the plasma membrane? TPC1 (Two-Pore Channel 1) on the tonoplast) or possibly carriers of the HKT (High-Affinity K^+ Transporters type in some species. At high external concentration, Na^+ could also enter the cell by borrowing K^+ carriers KUP/HAK (K^+ uptake/High-Affinity $K^{+\cdot}$) type.

In the mature root areas of the majority of plants, a second concentric barrier to that formed by the endodermis is formed at the root periphery on the exoderm, subepidermal cell layer. The suberization of the exoderm would occur later during root development than that of the endoderm and would be accelerated in case of drought [40] or salt stress.

4.2. Structure–Function Relationship of Root and Salt Stress

The current data about root structure and function, as discussed above, indicate that sodium ions can take a direct apoplastic path from the outer medium to the xylem at several levels of the root because endodermal suberization is not yet in place in the young roots area, and leaks remain in secondary roots appearance, which induces a brief discontinuity of the endoderm [41]. The relative contributions of the apoplastic and symplastic pathways of Na^+ transport is therefore largely conditioned by root anatomy and are likely to alter according to plant species and soil salinity. The apoplastic pathway (also called apoplastic leak) could be predominant in Na^+ transport under salt stress conditions.

Studies carried out on rice have shown that there is a strong correlation between sodium transport and the apoplastic tracer. In two different lines of rice, one more tolerant to salt than the other, a significant difference between the proportions of sodium amount and accumulated PTS in their aerial parts was observed [42,43]. This phenomenon results from the fact that the Na^+ entrance into the rice is essentially by free migration in the apoplast up to the stele in spots where the endoplasmic barrier is not functional. This apoplastic leak could occur at the lateral root connection points, at root's

apex before complete differentiation of rhizodermis and endodermis, and even in mature areas with differentiated endoderm because of the inherent permeability of the parietal broad outline [44].

It has been shown in rice that the control of apoplastic leakage of Na^+ into the roots is a critical determinant of salinity tolerance. The addition in the culture medium of silicon in sodium silicate partially blocked the apoplastic pathway and considerably improved the growth and photosynthesis of rice plants under salt stress, especially in the GR4 variety [44,45]. This improvement is correlated with the reduction of the Na^+ concentration in plant aerial parts. Furthermore, the authors found that the addition of sodium silicate in the culture medium reduced the accumulation of Na^+ in the aerial parts of sensitive and tolerant varieties at the same level [44,45].

The apoplastic pathway importance in the overall balance of Na^+ inflow varies with species. Garcia et al. [46] estimated that the contribution of the apoplastic pathway is 10 times greater in rice than in wheat. Moreover, it is important to emphasize that halophytes have root anatomy that can limit the entry of Na^+ via the apoplastic pathway. Indeed, the Caspary band in halophytes is 2–3 times thicker than in glycophytes, and the inner layer of cortical cells in halophytes can differentiate to form the second endoderm [2]. In cotton, considered as salinity-tolerant plant among cultivated species, salinity also accelerates the formation of the Caspary band and induces the formation of an additional exodermal layer [47].

All these findings show that there is a correlation between plant tolerance to salinity and the ability to control the apoplastic influx of Na^+ into the roots. It is, therefore, possible to postulate that reducing apoplastic leakage in sensitive species such as rice is a strategy for increasing plant tolerance to salinity. In this perspective, it is important to write down that complete blockage of apoplastic leakage is not likely to significantly affect water inflow and nutrient ion uptake because this leakage contributes little (less than 6%) in rice) to the incoming flows in the roots [46,48]. Some authors have estimated that the apoplastic flow contributes to the xylem flow feeding in a proportion that cannot exceed 1 to 5% [49]. This means that, concerning K^+, the symplasmic transport ensures the essential translocation of this ion from soil solution to the xylem vessels of the stele.

5. Potassium Availability in the Soil and Its Absorption by Plants

K^+ is an important cofactor in many biosynthetic processes, and in the vacuole, it plays key roles in cell volume regulation [50].

The concentration of K+ in the soil solution is generally between a few tens of µmol. L^{-1} and a few mmol. L^{-1} (i.e., approximately 10 to 10^3 times lower than that of the cell). The roots are thus confronted with a wide concentration range and the plants possess transport systems allowing them to grow over concentration ranges of K^+, ranging from 10^{-6} to 10^{-1} mol. L^{-1} [51].

An enhancement of the absorption capacity of K^+ by the root is observed when the availability of this ion in the soil is limited [52]. In wheat, K^+ deprivation increases the high-affinity transport efficiency, without altering the characteristics of low-affinity transport. This type of response has also been observed in barley and ryegrass [53]. This reaction is not general, but there are many proteins involved in high-affinity potassium transport. However, in Arabidopsis, two proteins have been identified as the most important transporters in this process. Interestingly, one of these transporters, AtHAK5, is a carrier protein and is thought to mediate active transport of potassium into plant roots, whereas the other protein, AKT1, is a channel protein and likely mediates a passive transport mechanism with an increased affinity for K^+ under conditions of potassium limitation [54,55].

Several different natural phenomena could be involved in root absorption capacity enhancement observed in response to K^+ deficiency in the soil. An initial model to account for this response proposes an allosteric regulation of the absorption capacity in terms of the cytosolic concentration of K^+, resulting in an inhibition by "feedback" of the transporters when the availability of this ion in the area is high, leading to an increase in its concentration in the cytoplasm [56]. Under this model, the K^+ availability diminution in the area leads to a decrease of K^+ concentration in the cytoplasm, which would lift the allosteric inhibition of transport, thus causing absorption capacity

augmentation. Another hypothesis, non-exclusive of the previous one, is based on the observation of modifications of the membrane polypeptide equipment when the plants are cultivated in a weakly concentrated potassium area, confirming the installation of new transport systems in barley [57], especially high-affinity transporters in barley [58], wheat [59], and *Arabidopsis thaliana*, [55,60]. In *Arabidopsis*, studies using the patch-clamp technical revealed that K^+ deficiency increases the activity of IRK-type channels (inward rectifying K^+ channel). This augmentation may reflect a corresponding gene(s) expression enhancement or the existence of a post-translational regulation mechanism (e.g., by dephosphorylation). However, the physiological meaning of the channels activity stimulation—And thus of passive transport systems in response to K^+ concentration diminution in the area—Is unclear, even though it is possible that channels may participate in the absorption function from a relatively low external K^+ concentration. Membrane potentials have indeed been found to be negative enough to be able to involve channels in the influx of potassium from an external solution of which K^+ concentration is less than 10 μM [61].

6. Long-range Transport in Xylem and Phloem

6.1. Transport into the Xylem

The Na^+ content of the roots appears to be relatively constant during salt stress. This steady-state probably results in part from root cells' ability to discharge Na^+ in the external area. It also results from Na^+ translocation in the stele and xylem vessels to the aerial parts. The sodium levels of the xylem and phloem may alter during the flow of plant sap. An increase of Na^+ concentration in xylem sap has been described in an "includer" type plant (definition below) *Plantago maritima* [48].

In opposition to this, a decrease of Na^+ concentration in xylem sap has been reported in "excluder" plants type—The sodium contained in the xylem is reabsorbed by roots during the ascent of the sap, and re-excreted toward the outside environment [48]. The amount of sodium that reaches the leaves via xylem sap can be controlled during transport in xylem vessels.

Unfortunately, there is a lack of knowledge about the mechanisms of Na^+ transport in the xylem. However, in *Arabidopsis* under moderate salt stress conditions (40 mM NaCl), *Sos1* mutants (having lost an H^+/Na^+ antiport system) accumulate fewer Na^+ in the aerial parts than wild-type plants [34,62]. This suggests that SOS1 plays a role in the transport of Na^+ from the roots to the aerial parts. However, the use of a reporter gene reveals that in the roots, SOS1 is expressed preferentially in the parenchymal cells around the xylem vessels [62]. Together, these data suggest that SOS1 has been involved in Na^+ secretion in xylem sap from stele parenchymal cells under moderate salt stress conditions.

In some plants, there is a reduction of Na^+ accumulation in the aerial parts. This reduction could be explained by sodium removal from the xylem before it reaches the foliar system. The existence of this strategy in plants has been clearly demonstrated by the research work of Adem et al. [63]. The authors have shown that in barley, the Na^+ concentration of the xylem sap varies together with the stem height (10 mM at the base of the stem and only 2 mM at the 8th leaf). This difference of concentration is important particularly for maintaining the photosynthetic activity of young leaves, which in return allows the formation and growth of new leaves. Molecular mechanisms of Na^+ removal from xylem sap ("desalting" of xylem sap) are beginning to be documented. In particular, the genetic analyses revealed that two transporters of the HKT family, *AtHKT1* in *Arabidopsis* and *OsHKT8* in rice, are involved in this desalting process.

The majority of plants maintain a high K^+/Na^+ ratio in their aerial parts, so it appears that the selectivity to the benefit of K^+ is ensured during the secretion. The ions are excreted in the xylem bundles via xylem transfer cells that can promote, or delay, the efflux of Na^+ in this vessel. The control of the Na^+ concentration in the xylem can also be carried out all along the stem by reabsorption of the sodium in exchange of potassium in the raw sap by the parenchyma cells [3]. H^+-ATPases of the plasma membrane would ensure the energization of the various transports resulting in the exchange

of Na^+ against K^+. The H^+ gradient created by these pumps would allow the secretion of K^+ via an antiport H^+/K^+, and a uniporter of Na^+ would ensure sodium reabsorption.

Concerning potassium, the ions absorbed at the level of the plasma membrane of the root superficial cells (epidermal and cortical) are transported towards the tissues of the stele by diffusion from one cell to another through plasmodesmata (symplastic pathway). After migration beyond the endodermal barrier, the ions leave the symplasm crossing a second plasma membrane at the level of the last living cells that border the vessels (xylem parenchyma). Once in the apoplast stellar, the ions are driven by the centripetal flow of water to the vessels, and the convection flow of the raw sap (water and mineral units) carried by transpiration and/or root thrust then exports them to the aerial parts [64].

The inner position of xylem parenchyma cells in the root makes the electrophysiology analyses using microelectrodes difficult. As a result, the mechanisms of secretion of ions in the xylem have been less studied than the mechanisms of absorption. It has been acknowledged that the stela's tissues are not able to accumulate ions and that these ions, inflated at the entrance of the symplasm, passively diffuse to the vessels. This passive diffusion was thought to be the consequence of an oxygen deficiency in the central tissues of the root that results in cell depolarization [65]. The stele cells in hypoxic conditions were then unable to retain the ions. However, Zhu et al. have shown that aeration of root pivotal tissues allows cells sufficient oxygenation. CCCP instantly blocks efflux in the xylem of the $^{36}Cl^-$ accumulated in advance but not efflux to the area through the epidermis [66]. These results show that the CCCP affects the existing system at the level of the stele and not the one located in the cells of the epidermis. Since the 1970s, it has been clearly established that the ions efflux in the stellar apoplast depends on specific transporters located on the plasma membrane of xylem parenchyma cells. Several experimental data indicate that absorption and secretion are controlled separately.

In general, the secretion of nutrient ions in the stellar apoplast could in many cases be a passive phenomenon, catalyzed by channels. For example, in *Arabidopsis*, the SKOR potassium channel of the Shaker family plays an important role in K^+ secretion in xylem sap [67]. The knowledge at the molecular level on the mechanisms of secretion of nutrient ions in the xylem sap is, however, still rather small.

6.2. Transport into the Phloem

The growth and development of the plant require distribution of photosynthesis products. These molecules synthesized in the so-called "source" organs (mature leaves) must then be relocated to the growing organs and non-photosynthetic plant tissues (organs called "wells," young leaves, flowers, seeds, fruits, roots). This relocation requires selective long-distance transport, which is provided by the phloem system.

Data obtained from barley show that the sodium contents of xylem and phloem sap are altered throughout transport in the vessels of the aerial parts [68]. The sodium contained in xylem would be absorbed and stored into leaf cells during its movement, and there would also be a translocation of a part of the sodium from xylem to phloem in the leaf, so that the sodium concentration in phloem sap has increased, as it moves from the top of the leaf to its base. Foliar anatomy, particularly in the area of young veins, suggests that such a transfer could occur either directly from apoplast to symplasm of phloem cells, or by symplasmic transport from parenchymal cells [68]. This recirculation of ions from xylem to phloem thus makes it possible to significantly reduce the salt content of the leaves. This has also been observed in some species such as Lupin [69], pepper [70], corn [71], and barley [13].

Perez-Alfocea et al. [72] have found that Na^+ translocation in the phloem of *Lycopersicon pennellii*, a wild type tomato that is tolerant to salinity, is more important than that observed in domesticated tomatoes. This suggests that the translocation of Na^+ into the phloem would be an adaptation strategy in plants. However, the Na^+ translocation direction and the conditions under which it occurs are probably critical. Indeed, it seems crucial that translocation by phloem does not transport Na^+ to the young tissues—Otherwise, it would completely inhibit their growth. In other words, translocation by the phloem should essentially redirect Na^+ to the roots. In the pepper plant, it has been shown that the translocation of Na^+ from the aerial parts to the roots only occurs when Na^+ is removed from the

nutrient solution, i.e. when there is a favorable gradient between phloem and roots [70]. In *Arabidopsis*, it has been shown that the sodium transporter *AtHKT1*, expressed in phloem tissues, assure Na^+ recirculation from the leaves to the roots through phloem by removing Na^+ from the rising stream of raw sap at the aerial parts. This system thus plays the role of controlling the Na^+ accumulation in the leaves and plant resistance to salt stress [73].

With regard to potassium, the phloem loading and its discharge contribute to the establishment of the osmotic potential gradients (and therefore hydric) created between the source organs (high concentration of sugars and ions in the phloem sap) and the well organs (lower concentrations). The osmotic gradient is initiated at the level of the source organs by the creation of an electrochemical potential due to the H^+-ATPases activity of the fellow cells that are in direct electrical contact with the cells of the screened canals (making the phloem vessels) via plasmodesmata. This energization of the membrane allows the influx of sugars (essentially sucrose) and potassium into the cells. In summary, the available data indicate that control of K^+ transport in phloem tissues of source and well organs contribute to three main functions: (i) the phloem cells membrane potential regulation, tending to bring its value closer to that of equilibrium potential of K^+ (E_K), (ii) the installation of the osmotic gradient responsible for the sap flow between the source and well organs, and (iii) well organs (including seeds and fruits) potassium supply.

The electrophysiological characterization of the potassium conductance of phloem cells is still poorly advanced because of the difficulty to obtain protoplasts. This difficulty is less with corn roots, whose stele is easy to separate from the cortex. Phloem cells can then be obtained by dissection in which potassium conductance has been identified. They are close to the IRKs in their selectivity and responses to inhibitors, but they show a small correlation. It means that they allow an entrance or output of potassium according to the membrane potential value. In *Arabidopsis*, the AKT2 gene from the Shaker potassium channel family could code this type of conductance [74,75].

In *Arabidopsis thaliana*, the use of plants expressing the GFP gene under the AtSUC2 gene promoter (active specifically in phloem fellow cells) made it possible to isolate protoplasts from these cells and to identify two potassium conductance—An outgoing conductance of the ORK type and an incoming conductance of the IRK type. However, conductance that may be specific to cells located either in the source regions or in the well regions has not yet been demonstrated.

7. Adaptive Strategies of Plants to Na^+: Exclusion and Inclusion

The ability of a plant to compartmentalize Na^+ at the cellular level induces a difference of Na^+ management in the whole plant. We can distinguish two ways of plants responses to salt (exclusion and inclusion). "These strategies characterize behavior patterns that are not mutually exclusive" (Levigneron et al. reviewed in [76]). Excluder type plants are generally salinity-sensitive and are unable to control the level of cytoplasmic Na^+. This ion is transported in the xylem, conveyed to the leaves by transpiration stream, and then partly "re-circulated" by the phloem to be brought back to the roots. These sensitive species, therefore, contain little Na^+ in the leaves and an excess in the roots. Includer plants, which are resistant to NaCl, accumulate Na^+ in the leaves where it is sequestered (in the vacuole, foliar epidermis, and old limbs). However, excluder plants also accumulate Na^+ in the vacuole of root and stem cells. Of course, these two types of behavior are extreme, and some species can incorporate behaviors characteristic of both types of strategy.

7.1. K^+ and Na^+ Transport Systems in Plants

The kinetic characteristics of K^+ transport systems were studied (since 1950) using the ^{42}K and ^{86}Rb tracers, in particular by Epstein et al. The incorporation rate analysis of the tracer into the excised barley roots, in terms of the external concentration, reveals complex kinetics that presents two phases [77]. This kinetics, which can be analyzed according to the Michaelis-Menten formalism, suggests the existence of two absorption mechanisms. The first mechanism corresponds to a high-affinity saturable system (Km $\approx$ 20 µM), which allows the influx of K^+ from low concentrations in the area (less than

1 mM). The second mechanism corresponds to a low-affinity system (Km ≈10 mM) responsible for ions absorption from high concentrations. The second absorption mechanism differs from the first by the fact that it is not selective for K^+ (vis-a-vis of Na^+), and its ability to transport K^+ depends on the nature of the accompanying anion [78]. Electrophysiological data obtained on roots suggest that H^+-K^+ symports are responsible for high-affinity K^+ transport [79]. Low-affinity absorption is passive and involves channels.

For sodium, it is established that its initial entrance from the external environment into the cytoplasm of the roots cortical cells is passive [48], either via non-selective voltage-dependent cation channels (NSCCs) [2] or probably via some family members of sodium transporter [80,81] (Figure 2).

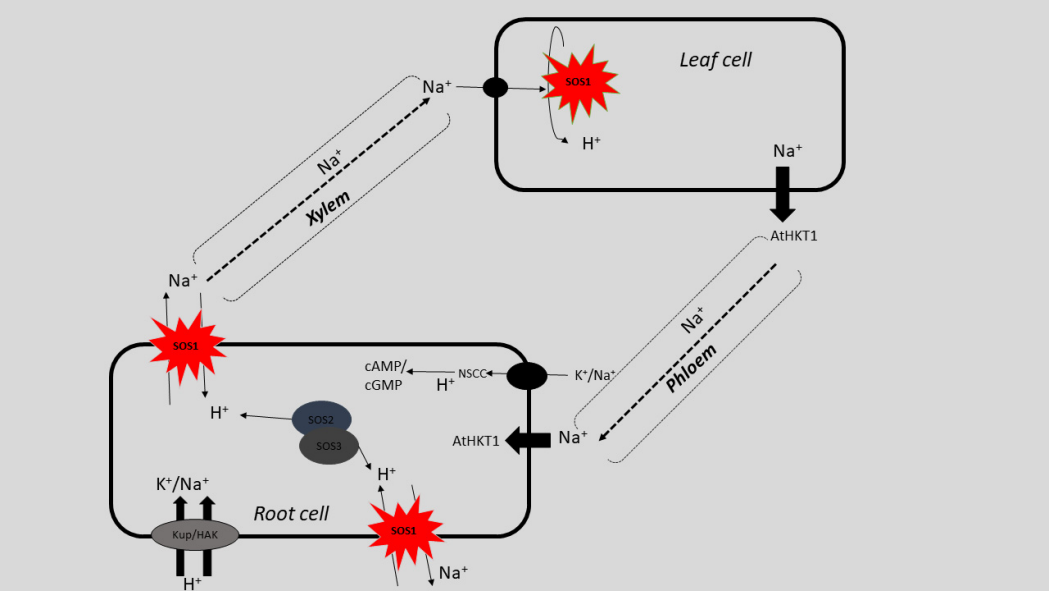

Figure 2. Na^+ transport at the level of the whole plant. Sodium ions can enter the cells of the root through non-selective channels (NSCCs) not formally identified at the molecular level, some of which appear to be inactivated by cyclic nucleotides (cAMP and cGMP; Maathuis and Sanders 2001), transporters HKT and high concentration of Na, KUP/HAK carriers. Na excretion cell roots to the soil solution or to the vessels of the xylem involve the antiport H^+/Na^+ SOS1, whose activity is regulated by the SOS3 CBL protein associated with the SOS2 kinase of the HKT conveyors allow desalinization of xylem sap and phloem loading in Na^+ at the leaf level.

Several families of channels and transporters involved in K^+ and Na^+ transport have been identified at the molecular level in plants.

7.2. Channels

7.2.1. Shaker Channels

These channels exist in plants, fungi, bacteria, and animals. The first members of this family were identified in animals.

These channels are formed with four subunits, which are organized around a central pore. The hydrophobic region of each subunit includes six transmembrane segments (TMS). A membrane loop (called P, for pore) between the fifth and sixth TMS participates in wall constitution of the central pore. Subunits can gather into homotetramers or heterotetramers. These channels are all voltage-regulated and active on the plasma membrane. They are very selective of K^+ beside Na^+. In higher plants, several Shaker channels have been cloned and characterized. There are nine members in *Arabidopsis*, with different functional properties, expression patterns, and localization [82]. The first two Shaker channels identified in plants are AKT1 and KAT1, cloned in 1992 in *Arabidopsis* [83].

In a very interesting way, the characterization of these functional systems has shown that they act as inward rectifying channels [55,84], despite strong homology with voltage-dependent, highly selective K^+ channels, which act as outgoing channels. This observation has generated a lot of interest and triggered numerous studies on the structure–function relationship of these channels, with the main objectives of understanding the mechanisms of opening-closing of the pore and the regulation by the voltage.

There are three main functional types of Shaker channels—incoming rectification channels (KAT, AKT1, and ATKC1 families), outgoing rectification channels (SKOR family), and low rectification channels (AKT2 family). The fourth TMS, carrying positively charged residues (R or K), is the cause of the channel sensitivity to the voltage. The pore loop (P) studies by controlled mutagenesis have identified a motif (TxGYG) involved in ionic selectivity.

The role in the plant of several *Arabidopsis* Shakers was analyzed by reverse genetics. In a general way, these channels allow the massive exchanges of K^+ (influx or efflux), between the symplast and the apoplast (K^+ entrance of the cell for the incoming channels, exit for the outgoing channels), entry, and exit by low rectification channels. They play a role in the removal of K^+ from the soil solution (AKT1 and AtKC1 channels in Arabidopsis), long-distance K^+ transport in the xylem and phloem (SKOR and AKT2 channels), or in the transport of K^+ in the guard cells at the origin of stomatal movements (GORK, KAT1, and KAT2 channels) [85,86]. The shaker channels, outstanding the potassium conductance of the plasmalemma, participate in parallel to the regulation of cellular potassium concentration, to the control of the membrane potential, and to the osmotic potential regulation.

7.2.2. KCO Channels

KCO (or TPK) is the second family of specific K^+ channels identified in plants. These channels are probably composed of either two subunits (family KCO-2P) or four subunits (family KCO-1P), which are organized around a central pore associating four domains (P for pore). In *Arabidopsis*, the KCO-2P family (two P domains per subunit) has five members, and the KCO-1P family (one pore domain per subunit) has only one member [85]. The first member of the KCO-2P family, KCO1, was discovered in silico via the use of the highly conserved GYGD motif in Shaker channels [87]. It has been expressed in insect cells, where it acts as a selective channel of K^+. At the subcellular level, *AtKCO1* has been localized at the level of the tonoplast [61,88], suggesting that it plays a different role from that of the Shaker channels in transporting K^+ through intracellular membranes. Electrophysiological analysis of vacuolar currents on invalidated mutant kco1 suggests that KCO1 contributes to SV type currents, which are outgoing and slow vacuolar currents [61].

7.2.3. Non-Selective Cationic Channels (NSCCs)

These channels, less selective of K^+ than Shaker, have been characterized in different cell types [89] NSCCs include CNGCs and GLRs, which are still poorly characterized. Obviously, all transporters have not significant role in potassium or sodium uptake, thus recent studies on GLRs showed that their expression throughout the plant, open up the possibility that GLR receptors could have a pervasive role in plants as non-specific amino acid sensors in diverse biological processes [90]. There has been no progress in elucidating their role in potassium and sodium uptake for the last two decades.

An indication of the CNGCs involvement in Na^+ influx is that the addition of similar cyclic nucleotides in the environment inhibits Na^+ influx and non-selective cation channel activity [91]. In animals, CNGCs are non-selective cationic channels involved in signal transduction in response to different stimuli. They are permeable to Ca^{2+}, Na^+, and K^+ [92]. Activation of these channels leads to membrane depolarization and cytoplasmic calcium concentration enhancement, thereby activating the signaling pathways dependent on this ion. These channels have a similar structure to the Shaker-type voltage-dependent potassium channels, a hydrophobic domain formed by 6 TMSs (named S1 to S6), and a P domain forming the pore between the fifth and the sixth TMS. In their hydrophilic N- and

C-terminal ends, they have respectively a calmodulin binding domain (CaMBD) and a cyclic nucleotide binding domain (CNBD).

In plants, a family of ion channels homologous to CNGCs animal channels was identified in the late 1990s. The first cDNA encoding a channel belonging to this family was cloned in barley by screening an expression library by searching for calmodulin-interacting proteins and was named *HvCBT1* [93]. The second member of the family was isolated from tobacco by the same approach [94]. This cDNA, named *NtCBP4*, has 61.2% identity with *HvCBT1*. In *Arabidopsis*, 20 family members, named CNGC-1 to 20, have been identified in silico by sequence analogy [95].

CNGCs are a class of nonselective cation channels that are permeable to monovalent and divalent cations such as Na^+, K^+, and Ca^{2+} [89,96,97]. Although their down-regulation can prevent Na^+ uptake, it can potentially be concomitantly harmful to the plants, as the uptake of other elements will be compromised. However, in rice root, the downregulation of the rice (*Oryza sativa*) *OsCNGC1* contributed to the superior tolerance of the cultivar FL478 to salt stress [25], as it could avert toxic Na^+ influx, in contrast to the sensitive cultivar, in which the gene was up-regulated by salinity stress. Also, *Arabidopsis thaliana* null mutants, *Atcngc10*, were found to have enhanced growth under salt stress compared to wild-type plants [98]. Furthermore, Atcngc3 T-DNA insertion mutants showed an increase in tolerance to high levels of NaCl and KCl [99]. With regard to the correlation between CNGC down-regulation and stress tolerance, Mekawy et al. (2015) evaluated the relative tolerance of two rice cultivars, Egyptian Yasmine and Sakha 102. They observed that the greater tolerance of Egyptian Yasmine was partially attributable to the down-regulation of OsCNGC1, with the concomitant up-regulation of plasma membrane protein 3 (PMP3), a plasma membrane protein involved in the inhibition of excess Na^+ uptake at the level of the root [100].

Also, some observations show that, in *Arabidopsis*, the *AtCNGC1 and AtCNGC2* channels introduced into yeast expression plasmids appear to complement a defective yeast mutant for K^+ transport [95]. In tobacco, over-expression of *NtCBP4* confers transgenic plants nickel tolerance and tin hypersensitivity that decrease Ni^{2+} accumulation and increase Pb^{2+} accumulation [94]. Subsequently, it has been shown that NtCBP4 is expressed on the plasma membrane of tobacco cells [94]. The hypothesis is that *NtCBP4* would be a transport system (perhaps permeable to Ca^{2+}) allowing Pb^{2+} entry into the cell.

The data in planta on the function of a CNGC were obtained indirectly following genetic analysis on an altered *Arabidopsis* mutant in response to a pathogen [101]. This study has made it possible, for the first time, to highlight the involvement of a CNGC ion channel in a signaling pathway. In general, CNGCs are probably involved, like their homologs in animal cell signaling [89,102]. They would be permeable to monovalent and/or Ca^{2+} cations and regulated by cyclic nucleotides and calmodulin. In plant CNGCs, the cyclic nucleotide-binding domain and the calmodulin-binding domain are both located in the C-terminal cytoplasmic region, where they overlap slightly [102].

7.3. Transporters

The KUP/HAK/KT family. A transporter belonging to a new family of K^+ transport systems has been identified in *Escherichia coli* (KUP1) [103] and in yeast *Schwanniomyces occidentalis* (SoHAK1) [104]. The *SoHAK1*expression in a mutated strain of *S. cerevisiae* for K^+ uptake systems restored growth onto a low K^+ concentration environment [104], SoHAK1 seems to be a high-affinity K^+ transporter. The homologs in plants, named KUP, HAK, or KT (for "K^+ uptake," "High-Affinity K^+ transporter," and K^+ Transporter, respectively), form a large family containing at least 17 members in rice [105]. The structure of these transporters is poorly known. The hydrophobicity profiles suggest that they have 12 TMSs and a long cytoplasmic loop between the second and third segments.

In plants, the first gene of the HAK/KT/KUP family, named *HvHAK*, was cloned in barley by qRT-PCR, with corresponding primers to conserved regions of *E. coli* KUP1 transporters and SoHAK1 [58]. In *Arabidopsis*, the first members identified in the HAK/KT/KUP family were cloned by complementation of a yeast mutant [106] or by the search for homologous sequences to KUP1 and HvHAK in the data banks [60]. Overexpression of AtKUP1 and AtKUP2 cDNAs induces an $^{86}Rb^+$

influx in yeast or in *Arabidopsis* growth cells [60,106]. For AtKUP1, the absorption kinetics in terms of concentration shows a Michaellian style in the low concentration range (less than 100 µmol. L^{-1}), raising the kinetics associated with the mechanism I in roots [60,106]. This similarity suggested that KUP-type systems are responsible for active K^+ transport with high affinity in plant cells. However, the analysis of absorption kinetics by the AtKUP1 system as a function of K^+ concentration also reveals low-affinity transport activity [60]. In other words, the AtKUP1 system alone can generate biphasic absorption kinetics, which evokes the kinetics observed in the roots (mechanism I plus mechanism II). The duality of transport kinetics by AtKUP1 could reflect two different modes of operation for this system. No current was detected by heterologous expression of AtKUP1 in the Xenopus oocyte, and the transport mechanisms unable to be determined [60]. However, the K^+ influx generated by *HvHAK1* and *AtKUP1* proteins in yeast is inhibited by the presence of Na^+ in the environment [58,106]. The localization of *AtKUP1* gene expression analyzed by northern blot, has led to variable results in which, the mRNA is undetectable in the roots but present in the aerial parts [60], mainly expressed in roots [58,106] or undetectable throughout the plant [107]. These variations could be associated with differences in plant growth conditions. This would mean that the accumulation of *AtKUP1 mRNA* is highly dependent on environmental conditions.

By a classic genetic approach based on the search for altered mutants in absorbent hairs growth, the authors of reference [108] have isolated another family member, named *TRH1* or *AtKUP4*. The trh1 mutant shows a decrease in $^{86}Rb^+$ uptake. The phenotype of absorbent hair growth of the mutant plants is not restored when they are grown in an environment containing 50 mM of K^+. The high-affinity K^+ transporter function of *TRH1* has been demonstrated by the complementation of yeast mutant *trk1*. TRH1 is expressed in the roots and in the aerial parts. It could be involved in the absorbent hair formation by allowing the influx of K^+ necessary for the growth and the elongation of these cells.

In general, all these HAK/KT/KUP transporters are not sufficiently characterized at the functional level, because of difficulties in expressing them in a heterologous system (a few rare members, however, express themselves in the yeast *S. cerevisiae* and/or in *E. coli* bacteria). In plants, they are present in many cell types and seem to be found on both the plasma membrane and the vacuolar membrane [105].

7.3.1. HKT Transporters

HKT transporters have homologs in fungi (TRK) and bacteria. Their predicted global structure, based on sequence analyses, is similar to that of potassium channels (at 2 TMS) that exist for example in bacteria. The hydrophobic region of the HKT polypeptides comprises four repeats of the (1 TMS/1 P/1 TMS) module. In the functional protein, the four loops are arranged to form a central pore [109].

All HKT transporters characterized so far in plants are permeable to Na^+, and some are also permeable to K^+. The role of these transporters in planta of K^+ transport has not yet been clarified. Several studies have demonstrated the role of these systems in planta in the transport of Na^+ and revealed that HKTs are involved in the tolerance of plants to salinity.

The protein sequence of *TaHKT1* has about 20% homology with the TRK systems identified in yeast and its structure would integrate 10 to 12 hydrophobic regions likely to correspond to TMS.

The *TaHKT1* expression in the Xenopus oocyte causes an activated current by the addition of K^+ or other cations to the external medium. The intensity of this current increases when the pH of the external medium is lowered. However, the analysis of transgenic plants overexpressing *TaHKT1* did not make it possible to highlight a contribution of this system to the absorption function of K^+ by the root [110]. Subsequent analyses revealed a sensitivity of the transport to the presence of Na^+ in the area. These data suggested that *TaHKT1* would rather function as a high-affinity Na^+: K^+ symport for K^+ (ca = 10 µM), energized by the electrochemical gradient of Na^+ across the membrane [111], which is completely unexpected energy coupling mechanism in plants. Moreover, this type of operation is limited to conditions of low external concentration of Na^+.

When the Na^+ concentration is higher, the transport of K^+ by *TaHKT1* would be blocked and this system would function as a low-affinity Na^+ transporter (*Km* close to 5 mM) [111]. The physiological

significance of this result remains unclear since in vivo K^+-transport analyses in higher plants have never revealed Na^+-K^+ symport activity (e.g., the addition of Na^+ in the medium does not stimulate K^+ uptake).

The only member of the HKT family in *Arabidopsis*, orthologue of the wheat *TaHKT1* gene, has been identified and designated as *AtHKT1*. The expression of this gene in yeast strains lacking the Na^+ efflux system aggravates their sensitivity to Na^+, but it does not suppress K^+ transport deficiency in *trk1* and *trk2* mutants that have difficulty to absorb potassium [112]

When expressed in the *Xenopus* oocyte, *AtHKT1* exhibits strictly selective Na^+ transport activity, without any permeability to K^+. Similarly, *AtHKT1*expression does not complement a type of *E. coli* mutant unable to absorb K^+, which helps to show that *AtHKT1* carries only Na^+.

AtHKT1 is expressed in the vascular tissues of the root and the aerial parts, at the level of the phloem and the xylem parenchyma [113].

While the *AtHKT1* gene is unique in *Arabidopsis*, it is interesting to note that the HKT family in rice has 7–9 members, depending on the cultivars [114]. The analysis of the polypeptide sequences of the transporters encoded by these genes shows a rather significant difference between the members—apart from two pairs of highly homologous transporters (OsHKT3/OsHKT9 and OsHKT1/OsHKT2, 93 and 91% identity, respectively), the percentage of identity between the different transporters is between 40 and 50%. Nipponbare (japonica), *Ni-OsHKT2*, and *Ni-OsHKT5* probably do not encode functional transporters due to large deletions or the presence of "stop codons" in the reading frame. However, *OsHKT2* is identified in another cultivar (*indica*) and codes for a functional transporter, Po-OsHKT2 [115].

Localization studies by analysis of transformed plants with a promoter (GUS fusion) has shown that these two HKTs are expressed at the vascular tissue level. Specifically, all of the available data (including in situ hybridization analyses) reveal that *OsHKT1* is localized in foliar vascular tissue but also in the root cortex and endoderm [32], whereas *OsHKT8* is mainly localized at the level of the xylem parenchyma, in the roots and in the leaves [116].

The most detailed data at the functional level concerns OsHKT1. This system is one of the closest counterparts in rice of the first HKT characterized, TaHKT1 (wheat), which is a transporter of K^+ and Na^+ (*OsHKT1* and *TaHKT1* have 67% identity). *OsHKT1* has been characterized by three different teams, leading to conflicting results. Expressed in the Xenopus oocyte, OsHKT1 is described as a cationic transport system, with little discrimination with respect to the different alkaline cations [117], or as a very selective transporter of Na^+ [115]. Expressed in yeast, it is described either as a K^+ permeable transport system [117] or as a Na^+ transport system blocked by K^+ [114]. OsHKT1 expression in *S. cerevisiae* yeast mutants deficient for K^+ transport did not allow growth on medium poor in K^+ (0.1 mM KCl). The growth inhibition test on *S. cerevisiae G19* yeast strains, highly sensitive to Na^+ following the disruption of ENA genes (which code for Na^+ excretory ATPases), revealed that the cells expressing OsHKT1 exhibited more sensitivity to Na^+ than those expressing TaHKT1 in the presence of 50 and 100 mM NaCl.

7.3.2. CHX Transporters (Monovalent Cation H^+ Exchanger)

These transport systems have been identified in plants on the basis of their homology with systems previously characterized in other organisms, such as bacteria, yeasts or algae. Only transporters involved in sodium compartmentalization in the plant vacuole are now relatively well known.

As in unicellular organisms, transports through the tonoplast is activated by an H^+-ATPase pump that establishes a proton gradient [118]. The operation of the CHXs is electron-based and thus does not disturb the potential difference across the membrane. These systems are probably involved in both monovalent cation homeostasis and cytoplasmic and/or vacuolar pH regulation [119].

From a biochemical point of view, tonoplast antiport Na^+/H^+ activity, which may be involved in sodium vacuolar compartmentalization, was initially demonstrated by the Blumwald group in several species [120]. This Na^+/H^+ antiport activity was associated with a 170 kDa vacuolar protein identified

in *Beta vulgaris*, whose accumulation is increased by NaCl treatments [121]. Antibodies planned against this protein inhibited the Na^+/H^+ antiport activity. This protein was, therefore, a good candidate for the antiport activity detected on the tonoplast but its coding gene remains unknown.

From the molecular point of view, an *Arabidopsis* cDNA, named *AtNHX1*, related to the yeast ScNHX1 protein, constituted the first characterized system. Only this tonoplast antiport Na^+/H^+ of Arabidopsis antigen has yet clearly been involved in sodium vacuolar compartmentalization [5,120,122,123]. The expression of this plant cDNA complements defective yeasts in the Na^+/H^+ transporter present in the vacuolar membrane [123]. In *Arabidopsis*, *AtNHX1* overexpression confers to transgenic plant tolerance to external Na^+ concentrations above 200 mM [5]. *AtNHX1* is expressed in all plant tissues and is found on the internal system tonoplast and on the membranes (RER, Glogi). Systematic sequencing of the *Arabidopsis thaliana* genome has identified 35 genes that can encode proteins being similar to antiport Na^+/H^+. Constitutive overexpression of *AtNHX1* improves salinity tolerance also in tomato [124], *Brassica napus* [125], and soybean [126]. Fukuda et al. have identified an *AtNHX1* homologue in rice, *OsNHX1*. OsNHX1 expression is induced into the roots and into the aerial parts during salt stress. The authors found that OsNHX1overexpression enhances the salinity tolerance of transgenic cells and plants [127].

Within the *CHX* family, some members may be good candidates for K^+ transport. This is the case in *Arabidopsis* for AtKEAs that resemble the K^+/H^+ bacterial antigens KefB and KefC. However, no experimental data for these systems are available, except for expression data in *Arabidopsis* tissues. Of the 28 KEA genes in this plant, 18 are specifically expressed during the microgametogenesis phase or in sporophytic tissues, suggesting that CHXs are involved in the regulation of potassium homeostasis in the pollen growth phase and germination [128]. Two CHXs have been characterized in more detail. *AtCHX17* appears to be preferentially expressed in roots under stress conditions, such as high salt concentrations, low external pH, low external K^+ concentration, and/or basic acid treatment [125]. The analysis of the mutant *AND-T atnhk17* suggests that this gene has a function in potassium homeostasis since the mutant plants accumulate less potassium than the wild ones. When expressed in yeast, *AtNHX17* co-localizes with markers of the *Golgi apparatus* and complements the pH sensitivity of a *kha1* mutant yeast strain [129], suggesting a role in potassium homeostasis and pH regulation under stress conditions. Loss of function mutants of this gene showed alteration in the ultrastructure of the chloroplast with a sharp decrease in chlorophyll level in the leaves, and an increase in cytosolic pH in the guard cells. The growth of *atnhx23* mutants was enhanced by the addition of high concentrations of potassium in the environment but altered by the addition of NaCl [130]. All these data suggest that *AtNHX23* is an antiport K^+ (Na^+/H^+) active at the level of the chloroplast envelope and involved in potassium homeostasis and perhaps in regulating the pH of the stroma.

The Na^+/H^+ antiport systems of the plasma membrane are still poorly characterized. The only information relates to the SOS1 protein in *Arabidopsis*, which has a homologous sequence with antiport Na^+/H^+ and would be involved in sodium efflux at the plasmalemma level [62]. Evidence has been provided that SOS1 does have antiport Na^+/H^+ activity [62].

The sodium hypersensitive *Arabidopsis* mutant *sos1* exhibits, when cultivated in presence of moderate NaCl concentrations (40 mM), higher Na^+ content in its roots than those observed in the plant control of wild-type genotype. Moreover, using the reporter gene system, the authors have highlighted the localization of SOS1 in epidermal cells at the root end. These results suggest the involvement of *SOS1* in Na^+ efflux from the roots in the environment. In addition, it is interesting to note that SOS1 overexpression in *Arabidopsis* significantly improves plants tolerance to salinity. AtSOS1 is, therefore, an important determinant of salt sensitivity in plants. *AtSOS1* activity is controlled by *AtSOS2* and *AtSOS3*. AtSOS3 (a Ca^{2+} affine protein belonging to the CBL family) directly interacts with AtSOS2 which a serine/threonine protein kinase is [131]. The interaction of *AtSOS3 and AtSOS2* triggers *AtSOS2* protein kinase activity, which phosphorylates and activates SOS1. Moreover, CBL/CIPK perceive cytosolic Ca^{2+} signals resulting from salt stress and have important roles in regulating salt stress response and ion homeostasis [132].

7.4. Ion Transporters Mediating Role in Salt Tolerance

In Arabidopsis roots, *AtCNGC3* is thought to be involved in Na^+ fluxes. It has been reported that a null mutation in *AtCNGC3* would reduce the net Na^+ uptake during the early stages of NaCl exposure (40–80 mM). However, longer exposure of wild type (WT) and mutant seedlings to NaCl (80–120 mM), induces the accumulation of similar Na^+ concentrations in both plants [99].

These results indicate the involvement of *AtCNGC3* in Na^+ uptake during the early stages of salt stress. In salt-tolerant rice varieties, *OsCNGC1* is negatively more regulated than in salt-sensitive varieties subjected to salt stress conditions [133]. *Arabidopsis thaliana AtHKT1;1*, facilitates the influx of Na^+ into heterologous expression systems [134]. Apparently, there is a determinant of salt stress tolerance that controls the influx of Na^+ into the roots, resulting in lower accumulation of Na^+ in *athkt1* mutants than in WT plants [135]. Horie et al. demonstrated that *OsHKT2;1*, regulates the influx of Na^+ into root cells [32]. Plants lacking the *OsHKT2;1* gene, when exposed to 0.5 mM Na^+ in the absence of K^+, exhibit lower Na^+ accumulation and reduced growth [32]. *OsHKT2;2/1*, a new isoform of HKT isolated from the rice plant roots that is no more than an intermediate between *OsHKT2;1* and *OsHKT2;2*, was supposed to confer salt tolerance to the Nona Bokra rice cultivar by allowing the absorption of K^+ in roots under salt stress [136]. It has now been shown that OsHKT2;2/1 regulates the influx of Na^+ into the roots of plants exposed to salt stress [137]. Note that the constitutive overexpression of *AtNHX1* in Arabidopsis improves salt tolerance [138]. Besides, overexpression of NHX1 in various transgenic plants, such as Brassica [139], cotton [17], maize [140], rice [141], tobacco [142], tomato [143], and wheat [144], exposed to NaCl concentrations ranging from 100 mM to 200 mM improve their tolerance to salt stress. The induction of NHX1 and NHX2 in response to salt stress depends on ABA [145,146]. It is widely known that, during salt stress, NHX activity increases, which promotes salt stress tolerance in many plants [147]. AtCHX21, expressed in the endodermal cells of the roots and its mutants, subjected to salt stress, accumulate less Na^+ in their xylem and leaves sap, indicating that CHX21 could be involved in the transport of Na^+ through the endoderm in the stele [148]. Under moderately saline conditions, SOS1 most likely occurs in the xylem load of Na^+, due to the fact that Na^+ accumulates to a lesser extent in sos1 mutants [149]. In high salinity conditions, the xylem load of Na^+ is probably a passive process because a high concentration of cytosolic Na^+ in xylem parenchyma cells and a comparatively depolarized plasma membrane would favor the movement of Na^+ in the xylem [150]. Plants can recover xylem Na^+ from root cells to avoid high concentrations of Na^+ in aerial tissues [151]. This recovery has been observed in the basal regions of the roots and shoots of plants such as maize, beans, and soybeans [2,65]. In *Arabidopsis*, the *HKT1* mutation renders the mutants hypersensitive to salt stress and causes a greater accumulation of Na^+ in the leaves [152–154]. Inactivation lines have higher levels of Na^+ but low levels of K^+ in shoots. These results show that AtHKT1 is involved in the recovery of Na^+ from xylem while directly stimulating the load of K^+. This is one of the mechanisms to maintain a higher K^+/Na^+ ratio in shoots during salt stress in plants [155]. Synergistic effects of *SOS1*, *HKT1;5*, and *NHX1* have been proposed to regulate Na^+ homeostasis in *Puccinellia tenuiflora*, a halophytic plant [156]. The NaCl stress–induced vacuolar compartmentalization of its xylem load has been attributed to regulation by the differential expression of *NHX1* and *HKT1;5*. The NaCl stress-induced expression of SOS1 and NHX1 in the roots would also have been more effective in excluding Na^+ and Cl^- in the intertidal population of *Suaeda salsa* [157]. The genetic or environmental variation of salt tolerance among halophyte populations is related to the differential expression of Na^+ efflux channels. Detailed structural analysis of HKT1;5 was performed in *Triticum sp*. [158]. Variations in its amino acid sequences result in a change in Na^+ affinity and a subsequent change in salt tolerance in two species of *Triticum*. Comparative analysis of antioxidant mechanisms in *Cynodon dactylon* (salt-tolerant grass) and *Oryza sativa* (salt-sensitive plant) was corroborated by the high expression levels of SOS 1 and NHX1 transporters in Cynodon [159]. Salt tolerance in barley has been attributed to the regulation of Na^+ loading in root xylem elements [160]. This is controlled by a cross between reactive oxygen species (ROS), nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), Ca^{2+}, and K^+.

8. Calcineurin B–Like Proteins (CBL) and CBL-Interacting Protein Kinases (CIPK) and Salt Tolerance in Plants

Calcium serves as a pivotal messenger in many adaptation and developmental processes. Cellular calcium signals are detected and transmitted by sensor molecules such as calcium-binding proteins. In plants, the calcineurin B-like protein (CBL) family seems to be a unique group of calcium sensors and plays a key role in decoding calcium transients by specifically interacting with and regulating a family of protein kinases (CIPKs) [161]. Several CBL proteins appear to be targeted to the plasma membrane by processes of dual lipid modification by myristoylation and S-acylation. Additionally, CBL/CIPK complexes have been identified in other cellular localizations, suggesting that this network may confer spatial specificity in Ca^{2+} signaling.

Molecular genetics analysis of loss-of-function mutants involves various CBL proteins and CIPKs as important components of abiotic stress responses, hormone reactions, and ion transport processes. The event of CBL and CIPK proteins appears not to be restricted to plants, raising the question about the function of these Ca^{2+} decoding components in non-plant species.

8.1. Organization of the CBL–CIPK Network

CBL proteins have been initially identified from *Arabidopsis thaliana* [162]. Bioinformatics and comparative genomic analysis in plants have provided details about the sequence specificity, conservation, function, and complexity, and ancestry of CBL and CIPK proteins families from lower plants to higher plants. Bioinformatics research reports showed that *Arabidopsis thaliana* has 10 CBLs and 26 CIPKs [163], while in other plants, *Populus trichocarpa* has 10 CBLs and 27 CIPKs [164], *Oryza sativa* has 10 CBLs and 31 CIPKs [165], *Zea mays* has 8 CBLs and 43 CIPKs [165], *Vitis vinifera* has eight CBLs and 21 CIPKs [166], *Sorghum bicolor* has 6 CBLs and 32 CIPKs [166], *Glycine max* has 52 CIPKs [62], and *Brassica rapa L.* (Chinese cabbage) has 17 CBL genes [167].

All CBL proteins share a rather conserved core region consisting of four EF-hand calcium-binding domains that are separated by spacing regions encompassing an absolutely conserved number of amino acids in all CBL Proteins [161].

In contrast to CNB from animals and fungi, CBLs do not interact with a PP2B-type phosphatase that appears to be absent in plants.

Instead, CBL proteins interact with a group of serine-threonine protein kinases that evolutionary belong to the superfamily of CaM-dependent kinases (CaMKs) and form a phylogenetically separate cluster within the group of SNF1 related kinases. Therefore, this group has also been indicated as *Snf1* related kinase group 3 (*SnRK3*; [168]. As in other kinases of the CaMK group, the kinase domain in CIPKs is segregated by a domain called "junction domain" from the less-conserved C-terminal regulatory domain. Within the regulatory region of CIPKs, a conserved NAF domain (designated according to the prominent amino acids N, A and F) mediates binding of CBL proteins and simultaneously functions as an auto-inhibitory domain [169]. Binding of CBLs to the NAF motif removes the auto-inhibitory domain from the kinase domain, thereby conferring auto-phosphorylation and activation of the kinase [170]. Additional phosphorylation of the activation loop within the kinase domain by a yet unknown kinase further contributes to the activation of CIPKs [171].

Kinases related to CIPKs, like the AMP-activated protein kinase (AMPK), are dephosphorylated by type 2C protein phosphatases (PP2C) [172]. Interestingly, CIPKs can associate with PP2Cs like ABI1 and ABI2 via a C-terminal protein-phosphatase interaction (PPI) domain [173]. Currently, it is not known if PP2Cs may dephosphorylate CIPKs or if phosphorylation of PP2Cs by CIPKs occurs in vivo. Alternatively, the generation of CIPK/PP2C complexes could serve the formation of signaling kinase/phosphatase modules allowing for rapid alternating phosphorylation/dephosphorylation of target proteins.

In this regard, crystallization studies of CBL4 in complex with the regulatory domain of CIPK24 suggest that either CBLs or PP2Cs may mutually exclusively interact with the regulatory domain of CIPKs, and that formation of a trimeric complex is unlikely [174]. Therefore, it is tempting to speculate

that PP2C interaction with the PPI domain of CIPKs leads to competitive replacing of the CBL protein, which binds to the NAF and partly to the PPI domain. Dissociation of the CBL protein would release the otherwise masked auto-inhibitory domain of the CIPK resulting in inactivation of the kinase. Alternative Ca^{2+}-dependent binding of CBL proteins to the CIPK would favor phosphorylation of a given substrate by out-competing the PP2C from the complex. However, as the target stowage domains are still unknown for CIPKs and PP2Cs, such models can currently not consider the influence of substrate binding.

Interestingly, the PPI domain was shown to be structurally related to the kinase-associated domain1 (KA1) of the kinase KIN2/PAR-1/MARK subfamily [174,175]. Moreover, SnRK1, the SNF1 homologous in plants, also contains such a structural domain [175]. Although the function of this domain is not known, this finding may point to a mechanism of protein regulation that is conserved from animals to plants [174,175].

8.2. Mechanisms of CBL-CIPK Pathway

Structural characteristics of CBL and CIPK proteins provide the basis for their interaction. The crystal structure of the complex of Ca^{2+}-CBL4 with the C-terminal regulatory domain of CIPK24 was first resolved [174]. It reveals how the CBL-CIPK complex decodes intracellular Ca^{2+} signals provoked by extracellular stimulation [176]. The CBL protein harbors four elongation factor hands (EF-hands), and each EF-hand contains a conserved α-helix-loop-α-helix structure responsible for Ca^{2+} binding [163]. The EF-hands are organized in fixed spaces that are 22, 25, and 32 amino acids distant from EF1 to EF4 in turn [177,178]. The loop region is characterized by a consensus sequence of 12 residues DKDGDGKIDFEE [163]. Amino acids in positions 1 (X), 3 (Y), 5 (Z), 7 (−X), 9 (−Y), and 12 (−Z) are responsible for Ca^{2+} coordination [176]. EF1 contains an insertion of two amino acid residues between position X and position Y. Variation of amino acids in these positions causes the change of Ca^{2+}-binding affinity [163]. Amino acid residues of CBL4 at positions X, Y, Z, and −Z bind Ca^{2+} depending on side-chain donor oxygen, while backbone carbonyl oxygen atom and water facilitation are used at positions −Y and −X, respectively [176].

The CIPK protein consists of two domains, one is the conserved N-terminal kinase catalytic domain, which comprises a phosphorylation site-containing activation loop, and the other is the highly variant C-terminal regulatory domain harboring NAF/FISL motif and a phosphatase interaction motif (PPI) [170]. The NAF motif, named by its highly conserved amino acids Asn (N), Ala (A), Phe (F), Ile (I), Ser (S), and Leu (L), is necessary for binding CBL protein. This motif is necessary for sustaining the interaction between CIPK24 and CBL4 and is able to attach the C-terminal regulatory domain of CIPK24 to cover its activation loop for keeping the kinase in an auto-inhibited state (Figure 3) [179]. Attachment of Ca^{2+} by EF-hands leads to the modification of molecular surface properties of CBL4 [176] and supports CBL4 interact with CIPK24 via the NAF motif. The interaction triggers the conformational changes of CIPK24 and exposes its activation loop [180]. Once the activation loop is free, the auto-inhibited CIPK24 is phosphorylated by an unknown upstream kinase and activates CIPK24. Subsequently, the activated CIPK24 phosphorylates the Na^+/H^+ exchanger SOS1 on the PM to exclude the excess Na^+ from the cell (Figure 3a) [180]. Abscisic acid-insensitive 2 (ABI2), a member of protein phosphatase 2C (PP2C), was identified as a CIPK24-interacting phosphatase [179]. The salt-tolerant phenotype of *abi2* indicated that ABI2 is a negative regulator of CIPK24 in the SOS pathway. Up to now, the blocking mechanism of ABI2 in the CBL4-CIPK24 pathway is not yet elucidated. It is assumed that ABI2 might function in the process of dephosphorylating SOS1 (Figure 3b) or CIPK24 (Figure 3c) [179].

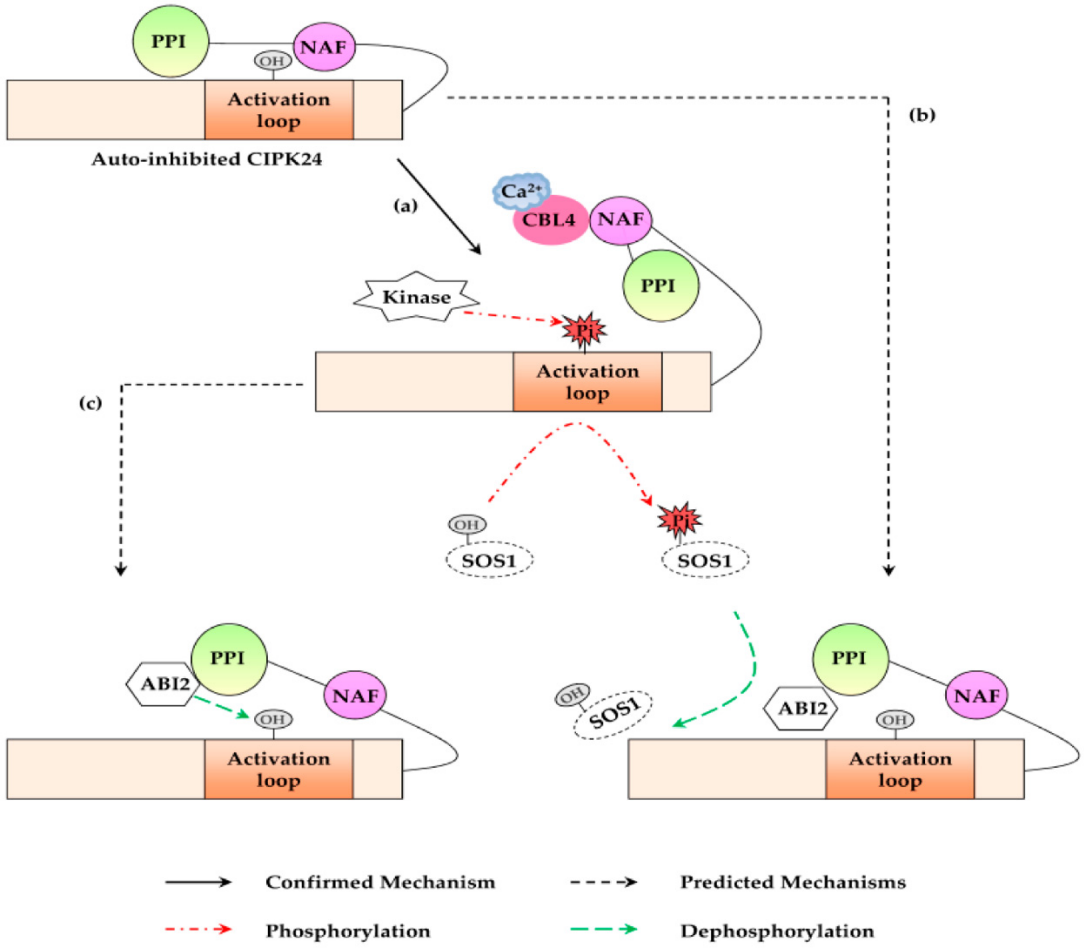

Figure 3. Mechanism of Calcineurin B-like protein 4 (CBL4)-CBL–interacting protein kinase (CIPK24) signaling pathway. **(a)** The Ca^{2+}-binding CBL4 interacts with the NAF motif of CIPK24 and changes the conformation of CIPK24. CIPK24 exposes its activation loop and then is phosphorylated by an unknown upstream protein kinase. Activated CIPK24 phosphorylates and stimulates salt overly sensitive 1 (SOS1), subsequently. **(b)** Abscisic acid-insensitive 2 (ABI2) binds to the phosphatase interaction (PPI) domain of CIPK24 and dephosphorylates SOS1 which was phosphorylated by CIPK24. **(c)** Activated CIPK24 is dephosphorylated by ABI2, and its activity is inhibited. (Adapted from Mao et al. (2016)).

8.3. Physiological Roles of CBLs and CIPKs in Plant Responses to Abiotic Stress Signals

The physiological roles of CBL and CIPK were firstly uncovered in salt overly sensitive (SOS) pathway (Figure 4) [180]. The *Arabidopsis* mutants *sos1*, *sos2*, and *sos3* produced the same salt-sensitive phenotype under high-salt stress [181]. SOS3 and SOS2, also known as CBL4 and CIPK24 respectively, were demonstrated to synergistically up-regulate the activity of plasma membrane (PM)-located Na^+/H^+ exchanger SOS1 in *Arabidopsis*, leading to the Na^+ efflux from cells in the high-salt environment [180].

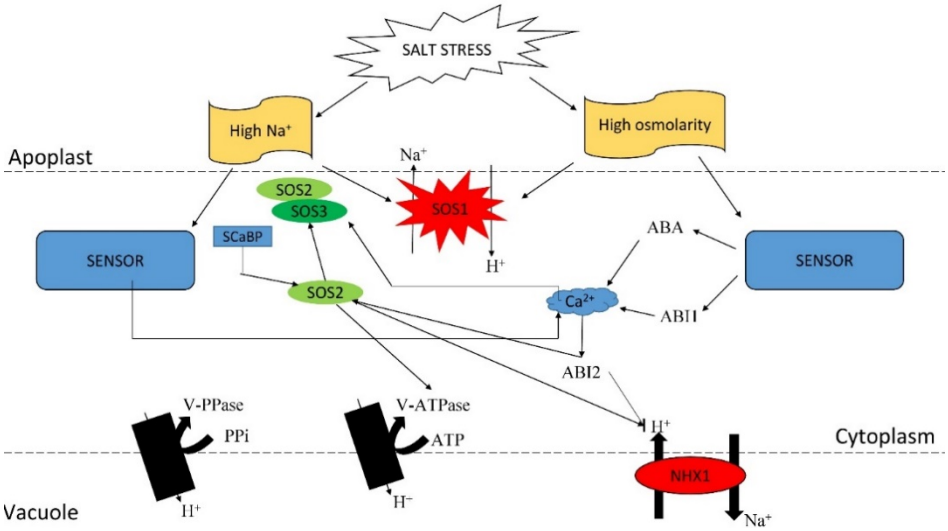

Figure 4. Signaling pathways responsible for Na^+ extrusion in Arabidopsis under salt stress. Excess Na^+ and high osmolarity are separately sensed by unknown sensors at the plasma membrane level, which then induce an increase in cytosolic Ca^{2+}. This increase is sensed by SOS3, which activates SOS2. The activated SOS3-SOS2 protein complex phosphorylates SOS1, the plasma membrane Na^+/H+ antiporter, resulting in the efflux of Na^+ ions. SOS2 can regulate NHX1 antiport activity and V-H+-ATPase activity independently of SOS3, possibly by SOS3-like Ca^{2+}-binding proteins (SCaBP) that target it to the tonoplast. Salt stress can also induce the accumulation of ABA, which, by means of ABI1 and ABI2, can negatively regulate SOS2 or SOS1 and NHX1.

It has been found that CBL-CIPK pathways work as regulators in nutrients transport systems, regulating sodium (Na^+) [180], potassium(K^+) [182], magnesium (Mg^{2+}) [183], nitrate (NO_3^-) [184], and proton (H^+) homeostasis [185]. Recently, in some reviews, particular attention to the possible involvement of the CBLs and CIPKs in different ions sensitivity has been drawn [186,187].

As calcium sensor relieves in plants, calcineurin B–like (CBL) proteins provide an important contribution to decoding Ca^{2+} signatures elicited by a variety of abiotic stresses. Currently, it is well known that CBLs perceive and transmit the Ca^{2+} signals mainly to a group of serine/threonine protein kinases called CBL-interacting protein kinases (CIPKs).

In the year 2016, Cho et al. reported that the CBL10 member of this family has a novel interaction partner besides the CIPK proteins. Yeast two-hybrid screening with CBL10 as bait identified an *Arabidopsis* cDNA clone encoding a TOC34 protein, which is a member of the translocon of the outer membrane of chloroplasts (TOC) complex and possesses the GTPase activity. Bimolecular fluorescence complementation (BiFC) analysis verified that the CBL10–TOC34 interaction takes place at the outer membrane of chloroplasts in vivo and thus decreases its GTPase activity in *Arabidopsis* [188].

These findings indicate that a member of the CBL family, CBL10, can modulate not only the CIPK members but also TOC34, allowing the CBL family to relay the Ca^{2+} signals in more diverse ways than currently known.

In tomato, the calcium sensor Cbl10 and its interacting protein kinase *Cipk6* define a signaling pathway in plant immunity [189]. Ca^{2+} signaling is an early and necessary event in plant immunity. The tomato (*Solanum lycopersicum*) kinase *Pto* triggers localized programmed cell death (PCD) upon recognition of *Pseudomonas syringae* effectors AvrPto *or* AvrPtoB. In a virus-induced gene silencing screen in *Nicotiana benthamiana*, Fernando and al. identified two components of a Ca^{2+}-signaling system, Cbl10 (for calcineurin B-like protein) and *Cipk6* (for calcineurin B-like interacting protein kinase), as their silencing inhibited Pto/AvrPto-elicited PCD. *N. benthamiana* Cbl10 and Cipk6 are also required for PCD triggered by other plant resistance genes and virus, oomycete, and nematode effectors and for host susceptibility to two *P. syringae* pathogens.

Tomato *Cipk6* interacts with Cbl10 and its in vitro kinase activity is enhanced in the presence of *Cbl10* and Ca^{2+}, suggesting that tomato Cbl10 and Cipk6 constitute a Ca^{2+}-regulated signaling module. Overexpression of tomato *Cipk6* in *N. benthamiana* leaves causes accumulation of reactive oxygen species (ROS), which requires the respiratory burst homolog *RbohB*. Tomato Cbl10 and Cipk6 interact with *RbohB* at the plasma membrane. Finally, Cbl10 and Cipk6 contribute to ROS generated during effector-triggered immunity in the interaction of *P. syringae* pv tomato DC3000 and *N. benthamiana*. The role of the Cbl/Cipk signaling module in PCD has been identified, establishing a mechanistic link between Ca^{2+} and ROS signaling in plant immunity [189].

Xu et al. showed that the protein kinase CIPK23, encoded by the Arabidopsis Low-K^+-sensitive 1 (*LKS1*) gene, regulates K^+ uptake under low K^+ conditions. Lesion of *LKS1* has reduced K^+ uptake and caused leaf chlorosis and growth inhibition, whereas overexpression of *LKS1* significantly enhanced K^+ uptake and tolerance to low K^+. They demonstrated that *CIPK23* directly phosphorylates the K^+ transporter *AKT1* and further found that CIPK23 is activated by the binding of two calcineurin B-like proteins, CBL1 and CBL9 [55]. Further research on protein kinase *CIPK23* in *Arabidopsis* has revealed that CIPK23 is expressed in a variety of cell types and tissues and regulates distinct physiological processes including the opening/closing of stomata in the leaves, and the potassium uptake in the roots [190]. In addition, the authors showed that CIPK23 kinase interacts and functions with both CBL1 and CBL9 calcium sensors, providing a molecular link between intracellular calcium fluctuations and the regulation of transpiration and nutrient uptake. CBL1 and CBL9 can both recruit CIPK23 on the plasma membrane, suggesting that CIPK23-CBL complexes associated with the plasma membrane modulate the membrane on which the target proteins are located, including the AKT1 potassium channel [190,191] by proteins phosphorylation. Cheong et al provided more information on the mechanistic aspects of calcium signaling by plants. According to their finds, the combination of CIPK23 with a specific set of other components in the guard cells results in the regulation of the stomatal response to ABA, while CIPK23 and another set of components in the root tissues participate in the regulation of potassium absorption. Since CIPK23 is also present in other tissues, such as vascular tissues of roots, stems, and leaves, the authors hypothesized that CIPK23 could also be associated with other components of these tissues, for example during long-distance transport and distribution of K^+ throughout the plant. They showed that the other components that interact with CIPK23 include the CBL1 and CBL9 calcium sensors that functionally overlap in regulating stomatal movement and K^+ uptake. It is possible that other CBLs may also interact with CIPK23 in regulating K^+ nutrition. Such selective and overlapping interactions can encode unique responses that are different from any CBL–CIPK interaction. Among the CBLs that regulate a specific CIPK in the same process, some may play a more dominant role than others. For example, the functions of CIPK23 in stomatal response and K^+ absorption appear to be primarily regulated by CBL1 and CBL9, each functioning in other processes by regulating other CIPKs [190,192].

Hashimoto et al. have identified a novel general regulatory mechanism of CBL-CIPK complexes in that CBL phosphorylation at their flexible C-terminus probably induces conformational changes that enhance specificity and activity of CBL-CIPK complexes toward their target proteins. The phosphorylation status of CBLs does not appear to influence the stability, localization, or CIPK interaction of these calcium sensor proteins in general. However, proper phosphorylation of CBL1 is absolutely required for the in vivo activation of the AKT1, K^+ channel by CBL1-CIPK23 and CBL9-CIPK23 complexes in oocytes [190,193]. Moreover, the authors have shown that, by combining CBL1, CIPK23, and AKT1, the reconstituted CBL-dependent enhancement of phosphorylation of target proteins by CIPKs in vitro. In addition, they reported that phosphorylation of CBL1 by CIPK23 is also required for the CBL1-dependent enhancement of CIPK23 activity toward its substrate.

Recent studies have uncovered the crucial functions of CBL-CIPK complexes in an increasing number of biological processes like salt tolerance, potassium transport, nitrate sensing, and stomatal regulation [194]. CBL proteins determine the cellular localization of their interacting protein kinases in vivo and are essential for the activity of the resulting CBL-CIPK complexes toward their target

proteins [55,184]. Despite the established importance of CBL-CIPK complexes in regulating the activity of ion channels and transporters like SOS1, AKT1, AKT2, and NRT1 [195], only very few target phosphorylation sites of CIPKs have been clearly identified. The occurrence of phosphorylation of CBLs by CIPKs appears not to be restricted to the model organism *Arabidopsis*.

In 2017, it was reported that *BdCIPK31*, a CIPK gene from *Brachypodium distachyon*, functions positively to drought and salt stress through the ABA signaling pathway [196]. Overexpressing *BdCIPK31* functions in stomatal closure, ion homeostasis, ROS scavenging, osmolyte biosynthesis, and transcriptional regulation of stress-related genes. In fact, it appears that transgenic tobacco plants overexpressing *BdCIPK31* presented improved drought and salt tolerance and displayed hypersensitive response to exogenous ABA [196]. Further investigations revealed that *BdCIPK31* functioned positively in ABA-mediated stomatal closure, and transgenic tobacco exhibited reduced water loss under dehydration conditions compared with the controls. *BdCIPK31* also affected Na^+/K^+ homeostasis and root K^+ loss, which contributed to maintaining intracellular ion homeostasis under salt conditions. Moreover, the reactive oxygen species scavenging system and osmolyte accumulation were enhanced by *BdCIPK31* overexpression, which was conducive for alleviating oxidative and osmotic damages. Additionally, overexpression of *BdCIPK31* could elevate several stress-associated gene expressions under stress conditions [196].

In 2013, *TaCIPK14* and *TaCIPK29* were found to confer single or multiple stress tolerance in transgenic tobacco [197]. Transgenic tobaccos overexpressing TaCIPK14 exhibited higher contents of chlorophyll and sugar, higher catalase activity, while decreased amounts of H_2O_2 and malondialdehyde (MDA), and lesser ion leakage under cold and salt stresses. In addition, overexpression also enhanced the seed germination rate, root elongation and decreased Na^+ content in the transgenic lines under salt stress. Higher expression of stress-related genes was observed in lines overexpressing *TaCIPK14* compared to controls under stress conditions [197].

Under conditions of high salinity, *TaCIPK25* expression was markedly down-regulated in wheat roots [198]. Overexpression of *TaCIPK25* resulted in hypersensitivity to Na^+ and superfluous accumulation of Na^+ in transgenic wheat lines. The *TaCIPK25* expression did not decline in transgenic wheat and remained at an even higher level than that in wild-type wheat controls under high-salinity treatment. Furthermore, the transmembrane Na^+/H^+ exchange was impaired in the root cells of transgenic wheat. These results suggested that *TaCIPK25* negatively regulated salt response in wheat [198].

9. Conclusions and Perspectives

The data available on the CHX family in *Arabidopsis* and other plants clearly highlight a novel and original mechanism involved in plants' tolerance to the salinity. This mechanism, which was previously not demonstrated in plants, allows detoxification of Na^+ in leaves by recirculation of this ion to the roots via the phloem. Plants face a dilemma regarding the transport of sodium. Sodium absorption is useful for lowering osmotic potential, being able to absorb water and maintaining turgor, but excess sodium is toxic. Many studies have focused on the toxic role of Na^+ in the plant during salt stress and the elucidation of the mechanisms of tolerance to this stress.

The role of Na^+ at lower concentrations is not well known. The current consensus is that the energization of the cell membrane is based solely on a proton gradient. However, the available data for some *CHXs* encourage us to continue to imagine that Na^+ (at non-toxic concentrations) can lead to symport systems and energize active K^+ uptake. Several indices seem to support this hypothesis, for example, AtNHX23 an antiport K^+ (Na^+/H^+) active at the level of the chloroplast envelope and involved in potassium homeostasis and perhaps in regulating the pH of the stroma. However, the Na^+/H^+ antiport systems of the plasma membrane are still poorly characterized. The available information is only related to the SOS1 protein in *Arabidopsis*, which has a homologous sequence with antiport Na^+/H^+ and would be involved in sodium efflux at the plasmalemma level. *SOS1* overexpression in *Arabidopsis* significantly improves plants' tolerance to salinity. AtSOS1 is,

therefore, an important determinant of salt sensitivity in plants. AtSOS1 activity is controlled by AtSOS2 and AtSOS3. AtSOS3 (a Ca^{2+} affine protein belonging to the CBL family) directly interacts with AtSOS2, which is a serine/threonine protein kinase.

Studies on CBLs and CIPKs over the past decade have greatly advanced our knowledge of the function of single proteins in distinct physiological processes. Major advances in understanding this signaling system were through the identification of an increasing number of targets regulated by the CBL-CIPK complexes. The progress of the research on the CBL and CIPK families in different plant species other than *Arabidopsis thaliana* is still at an infant stage; in most cases, it is limited to interaction studies and expression analysis of these families.

The CBL-CIPK signaling model emphasizes the importance of future research that focuses on the molecular mechanisms underlying the regulation of transporters that allow us to better understand plant's response to abiotic stress such as salt stress and also establish a proficient method of identifying molecular targets for genetically engineered resistant crops with enhanced tolerance to various environmental stresses. Therefore, the most important challenge for future research is not only functional characterization but also the elucidating of the details of synergistic functions in this interaction network and revealing the molecular mechanisms of the complexes regulating target proteins.

Author Contributions: Conceptualization, H.-Y.L. and T.K.; T.K. wrote this manuscript. X.-W.L., F.-W.W., K.F.I.C., and M.N. participated in the writing and modification of this manuscript. Validation, X.-W.L. All authors read and approved the final manuscript.

Funding: This research was supported by the Special Program for Research of Transgenic Plants (2016ZX08010-002), the National Natural Science Foundation of China (31601323), the National Key Research and Development Program (2016YFD0101005), and the Natural Science Foundation of Science Technology Department of Jilin Province (20170101015JC, 20180101028JC, 20190201259JC).

Acknowledgments: The authors are grateful to Prof. Li Haiyan for the critical discussion of this article.

Conflicts of Interest: The authors declare no conflict of interest.

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