Regulation of Cytosolic pH: The Contributions of Plant Plasma Membrane H\(^+\)-ATPases and Multiple Transporters

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Abstract: Cytosolic pH homeostasis is a precondition for the normal growth and stress responses in plants, and H\(^+\) flux across the plasma membrane is essential for cytoplasmic pH control. Hence, this review focuses on seven types of proteins that possess direct H\(^+\) transport activity, namely, H\(^+\)-ATPase, NHX, CHX, AMT, NRT, PHT, and KT/HAK/KUP, to summarize their plasma-membrane-located family members, the effect of corresponding gene knockout and/or overexpression on cytosolic pH, the H\(^+\) transport pathway, and their functional regulation by the extracellular/cytosolic pH. In general, H\(^+\)-ATPases mediate H\(^+\) extrusion, whereas most members of other six proteins mediate H\(^+\) influx, thus contributing to cytosolic pH homeostasis by directly modulating H\(^+\) flux across the plasma membrane. The fact that some AMTs/NRTs mediate H\(^+\)-coupled substrate influx, whereas other intra-family members facilitate H\(^+\)-uncoupled substrate transport, demonstrates that not all plasma membrane transporters possess H\(^+\)-coupled substrate transport mechanisms, and using the transport mechanism of a protein to represent the case of the entire family is not suitable. The transport activity of these proteins is regulated by extracellular and/or cytosolic pH, with different structural bases for H\(^+\) transfer among these seven types of proteins. Notably, intra-family members possess distinct pH regulatory characterization and underlying residues for H\(^+\) transfer. This review is anticipated to facilitate the understanding of the molecular basis for cytosolic pH homeostasis. Despite this progress, the strategy of their cooperation for cytosolic pH homeostasis needs further investigation.

Keywords: H\(^+\) transport proteins; cytosolic pH homeostasis; H\(^+\) transfer pathway; pH regulation

1. Introduction

As a fundamental activity in all living cells [1], cytosolic pH homeostasis is essential for the normal growth and stress responses of plants [2,3]. This is because basic cytosolic processes such as biochemical reactions, protein stability, ion channel/transporter activity, compartmental integrity, and membrane trafficking have strict pH requirements [1,4]. Simultaneously, most protein machineries (enzymes, motors, vesicle traffic, ribosomes, spliceosomes, assembly proteins, regulators, etc.) can only work within a narrow pH range [5]. Studies have shown that plant cytosolic pH is stable at a small range of 7.1–7.5 [5–8].

Cytosolic pH homeostasis is mainly controlled by the following three factors: first, chemical buffering components which comprise bicarbonate, phosphate, protein buffers (e.g., the imidazol group of histidine), etc. [9–12]; second, cytosolic H\(^+\) consumption and H\(^+\) generation by metabolism [5,8,13]; and third, the direct H\(^+\) flux across the plasma membrane and endomembrane [1,7,12,14–16].

In comparison with numerous reviews that concentrate on the organelle-located proteins which are responsible for H\(^+\) flux across the endomembrane [1,6,7], summaries...
regarding proteins that are directly involved in H\(^+\) efflux/influx across the plasma membrane are scarce, except regarding plasma membrane H\(^+\)-ATPases [14,17]. However, the topic with which we are concerned is not included in the two above-mentioned studies. Thus, this review focuses on seven types of proteins that possess direct H\(^+\) transport activity, namely, H\(^+\)-ATPase (H\(^+\)-pumping ATPase), NHX (Na\(^+\)/H\(^+\) exchanger), CHX (cation/H\(^+\) exchanger), AMT (ammonium transporter), NRT (nitrateg transporter), PHT (phosphate transporter), and KT/HAK/KUP (K\(^+\) transporter/high-affinity K\(^+\) transporter/K\(^+\) uptake permease), to summarize their plasma-membrane-located family members, the effect of changes in their transcript levels on extracellular/cytosolic pH, the H\(^+\) transport mechanism, and their functional regulation by either extracellular or cytosolic pH. Finally, prospects are presented in this field with emphasis on the necessity to determine the cooperative strategy of these proteins for cytosolic pH homeostasis.

2. Roles of Plasma Membrane H\(^+\)-ATPases and Multiple Transporters in Cytosolic pH Homeostasis

2.1. H\(^+\)-ATPase Family

2.1.1. Plasma-Membrane-Located Family Members, Function and the Effect of Their Expression Level Changes on the Cytosolic pH

Plant plasma membrane H\(^+\)-ATPases (H\(^+\)-pumping ATPase) have many family members. This notion is supported by the fact that 10 plasma membrane H\(^+\)-ATPases have been found in the model plant Arabidopsis genome [14,18,19]: 10 in rice [20], 12 in tomato [21], 4 in maize [22], 8 in Marchantia polymorpha [23], 10 in cucumber [24], 7 in potato [25], 9 in tobacco [26], and 13 in sunflower [27] genomes.

Plant plasma membrane H\(^+\)-ATPases actively pump H\(^+\) from the cytoplasm to the extracellular space using the energy generated by ATP hydrolysis; thus, they are essential for cytosolic pH homeostasis. The process of H\(^+\) efflux is not accompanied by other ions [17,28–30]. The effect of plasma membrane H\(^+\)-ATPases’ activity and/or expression level changes on the cytosolic pH is mainly reflected by the measurement of indirect extracellular pH variation. Firstly, pharmacological test results show that the addition of a strong H\(^+\)-ATPases activator, Fungal Toxin Fusicoccin, results in the acidification of tomato culture growth medium [31,32], whereas the inclusion of H\(^+\)-ATPases activity inhibitors (such as Erythrosin B or diethyl stilbestrol) leads to the alkalization of growth media [31]. Secondly, the expression of either single NpPMA2 (Nicotiana plumbaginifolia plasma membrane H\(^+\)-ATPase 2) or NpPMA4 (Nicotiana plumbaginifolia plasma membrane H\(^+\)-ATPase 4) in the heterologous yeast system leads to acidification of the growth medium [33]. Thirdly, in planta measurements through knockout and/or overexpression materials. Overexpression of an active isoform of AHA3 (T948D-AHA3, a mutant with T to D alternation at position 948 of Arabidopsis H\(^+\)-ATPase 3) enhances the tolerance of Arabidopsis to acid stress, a phenomenon which is consistent with its roles in the extrusion of toxic H\(^+\) from the cytoplasm [34]. Overexpression of rice OSA1 (Oryza sativa plasma membrane H\(^+\)-ATPase 1) leads to a ~1 unit decrease in the growth medium pH [35]. As two main H\(^+\)-ATPases in Arabidopsis roots [36,37], the single knockout of AHA2 (Arabidopsis H\(^+\)-ATPase 2) quantitatively results in a ~1 unit increase in the growth medium pH [37], and the single knockout of AHA1 (Arabidopsis H\(^+\)-ATPase 1) causes a 60% reduction in the H\(^+\) efflux capacity in planta [38]. The single knockout of AHA7 (Arabidopsis H\(^+\)-ATPase 7) also significantly reduces the H\(^+\) efflux capacity in the root hair zone under low-phosphorus stress [39]. All these results indicate the contribution of plasma membrane H\(^+\)-ATPases to cytosolic pH control, but the direct measurement of cytosolic pH changes upon their mutation/overexpression is still lacking. Until recently, the observation that the triple knockout of AHA6/8/9 (Arabidopsis H\(^+\)-ATPase 6/8/9) results in a ~0.5 unit decrease in the cytosolic pH has preliminarily quantified its role in cytosolic pH homeostasis [40].
2.1.2. Mechanism of H⁺ Transport

Results from AHA2 facilitate the understanding of the H⁺ extrusion pathway. It is proposed that a single, centrally located proton acceptor/donor (D684), an asparagine residue (N106), a positively charged arginine residue (R655), and a large central cavity form the H⁺ transporting unit [41]. The H⁺ transfer process can be divided into two steps: the H⁺ loading and release. Briefly, the side chain of a conserved D684 residue receives the proton, causing protonation of this residue. Then, the protonated form of D684 forms an occluded and hydrogen-bonded pair with the equally conserved N106 residue [29,42]. Subsequently, conformational movements trigger the opening of the proton exit cavity and the interruption of hydrogen bonding between N106 and D684, finally leading to proton release from D684 [41,42]. The conserved R655 is proposed to favor the release of the bound H⁺, possibly through polarizing the D684 side chain and modulating its pKa [41–43].

2.1.3. Regulation by Extracellular/Cytosolic pH

Activation of AHA7 occurs only when the extracellular pH is ≥6.0. Sensing of the extracellular pH is controlled by the extracellular loop between transmembrane segments 7 and 8 [44]. The relationship between the plasma membrane H⁺-ATPase and the cytosolic pH shows a “bell” shape, with the maximal transport activity occurring at around pH 6.5 [33,40,45,46]. For instance, the optimum pH for the H⁺-ATPase activity of a plasma membrane fraction from Arabidopsis is 6.6 [47], whereas the optimum pH for that from rice is 6.0 [48]. The observation that low pH treatment enhances the transport activity of plasma membrane H⁺-ATPase in rice and soybean under hydroponic conditions is interpreted as the result of cytosolic acidification [49,50]. In a wide pH range, a one-unit decrease in the extracellular pH would lead to a 0.1 reduction in the cytosolic pH [51,52]. As mentioned above, the cytosolic pH is generally 7.4, and the optimum pH for H⁺-ATPase activity is around 6.5. Thus, the cytosolic acidification caused by the low-pH treatment may enhance the activity of H⁺-ATPase by shifting cytosolic pH towards its optimum pH [53].

2.2. NHX Family

2.2.1. Plasma-Membrane-Located Family Members, Function and the Effect of Their Expression Level Changes on the Cytosolic pH

Amongst eight NHXs (Na⁺/H⁺ exchanger) in Arabidopsis [54,55], only two genes (AtNHX7 and AtNHX8) are located in the plasma membrane [56–59]. Homologues of the AtNHX7 widely exist in plants such as wheat, maize, and tomato [60–62]; however, no protein homologous to AtNHX8 has been found in the sequenced genomes of cereals [63]. The Arabidopsis AtNHX7/SOS1 (Arabidopsis thaliana Na⁺/H⁺ exchanger 7/Salt Overly Sensitive 1) functions as a plasma membrane Na⁺/H⁺ antiporter [56,64]. This protein mediates the efflux of Na⁺ out of the cytoplasm to the extracellular space, and exchanges equivalent H⁺ influx into the cytoplasm [7,65–67]. Thus, NHX genes are involved in cytosolic pH homeostasis [68,69]. Studies have demonstrated that the knockout of SOS1 in Arabidopsis and rice results in ~80% or ~40% reductions in the Na⁺/H⁺ exchange activity in plasma membrane vesicles, respectively, relative to activity in wild-type plants [70,71]. Upon NaCl treatment, the knockout of SOS1 reduces the capacity of H⁺ influx into the cytoplasm and results in cytosolic alkalinization [72,73].

AtNHX8 is proposed to function as a Li⁺/H⁺ exchanger [54]. Observations indicate that the knockout of AtNHX8 renders the plants more sensitive to Li⁺, whereas overexpression of this gene enables the plant to be more tolerant to Li⁺, confirming the contribution of this gene to Li⁺ extrusion [54]. However, direct experimental evidence involving H⁺ influx by AtNHX8 is still lacking.

2.2.2. Mechanism of H⁺ Transport

Interpretation of a lower resolution (25 Å) crystal structure of SOS1 demonstrates that it is a homodimer, which contains a membrane domain and an elongated, large, and
structured cytosolic domain [74]. To illustrate detailed mechanisms for H\textsuperscript{+} transport, higher-resolution structural techniques are necessary [74,75].

2.2.3. Regulation by Extracellular/Cytosolic pH

Knockout of the SOS1 transporter of *Physcomitrella patens* (PpSOS1) results in the enhanced influx capacity of Na\textsuperscript{+} at pH 4.5, but not for that at pH 9.0, suggesting that the transport activity of PpSOS1 is stimulated by low extracellular pH [76]. This acid-facilitated transport activity is in consistent with its Na\textsuperscript{+}/H\textsuperscript{+} antiport function.

2.3. CHX Family

Amongst 28 members of CHXs (c\textsuperscript{a}tion/H\textsuperscript{+} exchanger) in the Arabidopsis genome [77,78], AtCHX13 [79], AtCHX14 [80], AtCHX16 [81], AtCHX17 [81], AtCHX18 [81], AtCHX19 [81,82], and AtCHX21 [83] have been found to be localized in the plasma membrane. Three-dimensional homology modeling and point mutation results indicate that AtCHX17 has a core structure similar to Na\textsuperscript{+}/H\textsuperscript{+} antiporter [84]. It is thus proposed that AtCHX17 contributes to cytosolic pH homeostasis by mediating H\textsuperscript{+} influx across the plasma membrane. Expressions of AtCHX16–AtCHX19 in a yeast mutant defective in Na\textsuperscript{+} extrusion and K\textsuperscript{+}/H\textsuperscript{+} antiport rescue the alkaline pH-sensitive growth phenotype, also supporting their potential roles in cytosolic pH homeostasis [85]. However, to date, the H\textsuperscript{+}-coupled transport mechanisms of these plasma membrane CHXs have not been evidenced by direct experiments [7]. Notably, AtCHX13 is proposed to be a K\textsuperscript{+}-uptake transporter [79], but AtCHX14 is expected to be a K\textsuperscript{+}-efflux transporter [80]. This phenomenon suggests that the CHX intra-family may possess a distinct H\textsuperscript{+}-related transport mechanism, which should be assessed with caution.

2.4. AMT Family and NRT Family

2.4.1. Plasma-Membrane-Located Family Members, Function and the Effect of Their Expression Level Changes on the Cytosolic pH

Most AMTs (am\textsuperscript{m}monium t\textsuperscript{r}ansporters) reported thus far are localized in the plasma membrane [86]. Amongst four distinct transport mechanisms in the AMTs family, NH\textsubscript{3}/H\textsuperscript{+} co-transport and NH\textsubscript{4}\textsuperscript{+}/H\textsuperscript{+} symport are two mechanisms directly involving H\textsuperscript{+} transport [86]. Both TaAMT1;1 and AtAMT1;2 are NH\textsubscript{3}/H\textsuperscript{+} co-transporters [87,88], whereas PvAMT1;1 is a NH\textsubscript{4}\textsuperscript{+}/H\textsuperscript{+} symporter [89]. All three proteins are proposed to be localized to the plasma membrane [89–91]. Consistent with its role in H\textsuperscript{+} influx across the plasma membrane, the expression of PvAMT1;1 in oocytes leads to a ~0.12 unit decrease in cytosolic pH [89]. Correspondingly, the expression of an NH\textsubscript{4}\textsuperscript{+} uniporter (LeAMT1;1) in oocytes has no effect on cytosolic pH [92].

Although possessing different substrates, the substrate transport mediated by most NRTs (n\textit{it}rate t\textit{ransporters) shares a common feature, i.e., H\textsuperscript{+}-coupling [93–96]. Electrophysiological results demonstrate that plasma membrane nitrate transporters such as BnNRT1.2 [97], AtNRT1.1 [98,99], AtNRT1.4 [100], AtNRT1.5 [101], AtNRT1.6 [102], OsNRT1 [103], and OsNRT2.3b [104] mediate H\textsuperscript{+}/NO\textsubscript{3}\textsuperscript{−} symport, and the ratio of H\textsuperscript{+} is >1. Expression of OsNRT2.3b in oocytes leads to the ~0.12-unit acidification of cytoplasm [104]. In planta knockout of AtNRT1.1, the major molecular unit for nitrate uptake in Arabidopsis roots [105], causes a loss of alkalization of the growth medium and significantly reduces the adaptability of Arabidopsis to low-pH stress [106], supporting its contribution to cytosolic pH homeostasis by mediating H\textsuperscript{+} influx across the plasma membrane. AtNRT1.5 functions not only as a H\textsuperscript{+}/NO\textsubscript{3}\textsuperscript{−} symporter, but also as a K\textsuperscript{+}/H\textsuperscript{+} antiporter, mediating the efflux of K\textsuperscript{+} and an equivalent influx of H\textsuperscript{+} [107]. Both cases support its role in H\textsuperscript{+} influx, although the ratio of H\textsuperscript{+} is different (>1 for H\textsuperscript{+}/NO\textsubscript{3}\textsuperscript{−} symporter; =1 for H\textsuperscript{+}/K\textsuperscript{+} antiporter). Notably, substrate transport by some NRTs is not coupled to H\textsuperscript{+} [108], suggesting that not all plasma membrane NRTs confer H\textsuperscript{+} flux, a case similar to that of AMTs.
2.4.2. Mechanism of H⁺ Transport

PvAMT1;1 functions as a NH₄⁺/H⁺ symporter. H211E mutation results in the retaining of NH₄⁺ transport, but the loss of H⁺ transport in this protein. All these results demonstrate that H211 is necessary for H⁺ transport in PvAMT1;1 [89]. Mutations of Q67H and W145S lead to the uncoupling of H⁺ transport from NH₃/H⁺ transport in AtAMT1;2, indicating that the two residues (Q67 and W145) are essential for H⁺ transport in AtAMT1;2 [88].

The H⁺ transport in NRTs undergoes two steps. Firstly, proton receptor residues accept the proton in the outward-open conformation. Then, the transporters change into inward-open conformation and release H⁺ into the cytoplasm [109]. The crystal structure, in combination with mutation results, suggest that both the ExxER motif and a histidine residue confer H⁺ binding in plant NRTs [109–113]. AtNRT1.1 is the best structurally known plant NRT. Mutations of charged residues in its ExxER motif result in abolished H⁺ binding and NO₃⁻ transport [110,111]. The crystal structure of AtNRT1.1 demonstrates that, in the outward-open conformation, one H⁺ is bound by the ExxER motif, and the other H⁺ is bound by the H356 [114].

2.4.3. Regulation by Extracellular/Cytosolic pH

In agreement with their H⁺-coupled transport mechanism, extracellular acidification stimulates the transport activity of TaAMT1;1 [87] and PvAMT1;1 [89]. The observations from oocytes [115] and Arabidopsis mutants [105,106] indicate that the transport activity of AtNRT1.1 is enhanced by extracellular acidification. This extracellular-acid-stimulated transport seems a common feature of most plant plasma membrane NRTs, as detailed in Section 2.4.1 [97–104]. In contrast, a 0.16 pH unit (from 7.41 to 7.25) of cytosolic acidification arrests the nitrate transport activity of OsNRT2.3b. The amino acid residue H167 is necessary for this cytosolic pH regulation [104].

2.5. PHT Family

2.5.1. Plasma-Membrane-Located Family Members, Function, and the Effect of Their Expression Level Changes on the Cytosolic pH

Amongst five clades of PHT (phosphate transporters) family, PHT1 is conceived to be the only subfamily that is localized to the plasma membrane. PHT1 contains many family members. It is reported that 9, 13, 13, and 11 PHT1 proteins are found in Arabidopsis, rice, maize, and barley genomes, respectively [116–121]. Direct subcellular localization experiments confirm that at least AtPHT1;1 [122], AtPHT1;2 [123], AtPHT1;4 [123], At-PHT1;9 [124], OsPHT1;3 [125], OsPHT1;4 [35,126], OsPHT1;8 [127], HvPHT1;1 [128], and HvPHT1;6 [129] are localized to the plasma membrane.

PHT1 subfamily mediates Pi uptake from the soil, and its transport mechanism is conceived to be H⁺-coupled H₂PO₄⁻ symport; the ratio between H⁺ and H₂PO₄⁻ is 2:1 to 4:1 [117,120,130,131]. Although the H⁺-coupled HPO₄²⁻ (rather than H₂PO₄⁻) symport mechanism found in HvPHT1;6 challenges this consensus [129], the conclusion that substrate transport by PHT1 is coupled to H⁺ is unchanged. Consistent with its role in H⁺ influx across the plasma membrane, Pi uptake results in a ~0.2–0.3 unit decrease in cytosolic pH and corresponding alkalization of the growth medium in planta [132–134]. Expression of AtPHT1;9 in yeast leads to significant alkalization of the growth medium [124]. All these results indicate that PHT1 mediates H⁺ influx across the plasma membrane, and is finally involved in cytosolic pH homeostasis.

2.5.2. Mechanism of H⁺ Transport

The crystal structure of PiPT from Piriformospora indica reveals that the proton is first received by D324, then transferred from the proton transport pathway that is constituted by D45, D48, E108, R139, and D149 residues, and finally released to the cytoplasm [135,136]. Homology modeling and point mutant results demonstrate that D35, D38, R134, and D144 (corresponding to D45, D48, R139, and D149) are essential for H⁺ transfer in AtPHT1;1 [137].
2.5.3. Regulation by Extracellular/Cytosolic pH

When expressed in yeast, the transport activity of AtPHT1;1 is enhanced by extracellular acidification (pH gradually drops from 7.0 to 4.5) [137], whereas the activity of five rice PHT1 proteins exhibits a “bell-shaped” dependence on the extracellular pH. The optimum pH for the maximal transport activity is 6.5 in OsPHT1;1 [138] and OsPHT1;8 [127], 6.0 in OsPHT1;6 [139], and around 5.5–6.5 in OsPHT1;9 and OsPHT1;10 [140]. The difference in pH dependence amongst the above-mentioned PHT1 may be a result of the following. First, this distinct pH regulation strategy is an intrinsic property of PHT1. This is not surprising because even an H\(^+\)-independent transport mechanism has been reported in another type of Pi transporter, PHO1 (PHOSPHATE 1) [141]. Second, the fact that the transport activity of five rice PHT1 proteins under different pH conditions is measured by the yeast growth rate (OD\(_{600}\)), rather than direct Pi transport activity as shown in AtPHT1;1, may possibly cause an over-interpretation of the data. Thus, solid data from the direct Pi transport activity of PHT1 seem necessary for the clarification of their pH dependence.

2.6. KT/KUP/HAK Family

2.6.1. Plasma-Membrane-Located Family Members, Function and the Effect of Their Expression Level Changes on the Cytosolic pH

Plant KT/HAK/KUP (K\(^+\) transporter/high-affinity K\(^+\) transporter/K\(^+\) uptake permease) genes possess many family members. It is reported that 13, 27, and 27 KT/HAK/ KUP genes are found in the genome of Arabidopsis, rice, and maize, respectively [142–145]. At the protein level, most KT/HAK/KUP proteins are conceived to be localized to the plasma membrane [143,146,147]. AtKUP1-12 and AtHAK5 are the names of 13 Arabidopsis KT/HAK/KUP [144]. Experimental evidence shows that AtHAK5 [148], AtKUP2 [149], AtKUP4 [150], AtKUP6 [151], and AtKUP7 [152] from Arabidopsis, and OsHAK1 [153,154], OsHAK5 [155,156], OsHAK19 [154], and OsHAK21 [157] from rice, are localized to the plasma membrane.

The fact that the high-affinity uptake of K\(^+\) in Arabidopsis root protoplasts [158] and in barley roots [159] is H\(^+\)-coupled, and that AtHAK5 dominates the K\(^+\) uptake at less than 10 \(\mu\)M [148,160,161], indicate that AtHAK5 is most likely a K\(^+\)/H\(^+\) symporter in planta [160,162,163]. This deduction is partially supported by the results from homologous proteins NcHAK1 of Neurospora crassa [164,165] and DmHAK5 of Dionaea muscipula [166], which are conceived as K\(^+\)/H\(^+\) symporters, although further direct evidence is required (such as K\(^+\)- and H\(^+\)-dependent reversal potential shifts measured through electrophysiological experiments). Recently, crystal structure analysis of KimA (a plant KUP homologue) from Bacillus subtilis demonstrated that this protein functions as a K\(^+\)/H\(^+\) symporter [167]. Thus, HAK5, and even the HAK family, is conceived to mediate H\(^+\) influx across the plasma membrane, finally contributing to the cytosolic pH homeostasis. Overexpression of OsHAK5 in rice results in the pH elevation of the growth medium [168].

2.6.2. Mechanism of H\(^+\) Transport

The crystal structure, in combination with point mutation results, demonstrates that E233 confers H\(^+\) binding and release by its protonation and deprotonation in KimA (a plant KUP homologue from Bacillus subtilis) [167]. The conservation of this residue is expected to facilitate the understanding of H\(^+\) transport mechanisms in plant KT/KUP/HAK. Point mutation results show that the corresponding residue (E321) is essential for the transport activity of AtHAK5 [169].

2.6.3. Regulation by Extracellular/Cytosolic pH

Extracellular acidification significantly stimulates the transport activity of plant KT/HAK/KUP [166,170,171], which is consistent with its putative role in K\(^+\)/H\(^+\) symport.
3. Notable Issues in This Field

3.1. Not All Plasma Membrane Transporters Possess $\text{H}^+$-Coupled Substrate Transport Mechanisms, and Using Transport Mechanisms of a Protein to Represent the Case of the Entire Family Is Not Suitable

The observation that nutrient uptake by plants is co-transported with $\text{H}^+$ supports a long-standing hypothesis: transporters responsible for nutrient uptake are coupled with $\text{H}^+$ [158,159,172–174]. However, as a result of in-depth study of the molecular elements of nutrient ion transport, increasing evidence shows that not all ion transporters are $\text{H}^+$-coupled symporters and/or antiporters; examples are listed hereafter. First, four types of substrate transport mechanisms have been elucidated amongst AMTs [86]. Although $\text{H}^+/\text{NH}_3^+$ symport (represented by PvAMT1;1) and $\text{H}^+/\text{NH}_3$ cotransport (represented by AtAMT1;2) are two types of mechanisms that are coupled to $\text{H}^+$ [88,89], $\text{NH}_3$ transport (represented by AtAMT2) and $\text{NH}_4^+$ uniport (represented by LeAMT1;1) serve as another two types of mechanisms that are $\text{H}^+$-independent [175,176]. Second, regarding NRTs, although the majority of NRTs share a common feature, $\text{H}^+$-coupled transport, an exception was found for AtNRT2.4, which mediates $\text{H}^+$-uncoupled substrate transport [108]. Therefore, whether the transport is coupled with $\text{H}^+$ is not a common feature of one transporter family, but a special characterization of one protein. Attempts to clarify the transport mechanisms of all family members only through the functional analysis of a protein are unsuitable. Additionally, intra-family members possess distinct structural bases for $\text{H}^+$ transfer. For example, H356 is a key residue for $\text{H}^+$ binding in AtNRT1.1, but this residue is not conserved between AtNRT1.5 and AtNRT1.8 [110]. As a conserved residue amongst AMTs, H211 is necessary for $\text{H}^+$ transfer in PvAMT1;1. However, other intra-family members possessing this residue do not display similar $\text{H}^+$-coupled transport, as shown in PvAMT1;1 [89]. The variation in structural basis for $\text{H}^+$ transfer also indicates that $\text{H}^+$ transport is an individual issue of transporter proteins.

3.2. Special Caution Is Needed When Drawing Conclusion to the $\text{H}^+$ Transfer Mechanism of Transporters

The fact that transporter studies mainly focus on the transported ions, with less attention paid to the accompanied $\text{H}^+$, objectively leads to the inappropriate interpretation of $\text{H}^+$ transport. For example, first, several $\text{H}^+/\text{substrate}$ symport conclusions have been drawn just based on the observation that the transport activity of a protein is stimulated by extracellular acidification. Actually, functional enhancement by extracellular acidification may be the result of pH regulation. Second, $\text{H}^+$ transport conclusions have been obtained just based on the linkage of a protein functional property with the results of early physiological measurements (root or protoplast) also seem unreasonable. That is because physiological measurement reflects the whole situation, whereas transporters responsible for this physiological response possibly possess a distinct transport mechanism regarding $\text{H}^+$. Third, an $\text{H}^+$ symport mechanism is proposed by the original literature based on insufficient experimental results; however, subsequent reference citations strengthen this hypothesis and give it the appearance of a truth. All these are disadvantageous to the study of the transmembrane transport of $\text{H}^+$, which is an issue of physiological significance. Regarding the $\text{H}^+$ transport of a transporter, we believe it should be supported by the following evidence: (1) hydrogen isotope labeling tests for yeast, *Xenopus* oocytes, and plant genetic materials (knockout and/or overexpression) harboring the target gene, or direct $\text{H}^+$ flux measurements with technology such as non-invasive micro-tests, or extracellular/cytosolic pH measurements; (2) electrophysiological measurements. The pH regulation properties, as well as the reversal potential changes upon both the substrate and accompanying $\text{H}^+$ concentration variations, should be contained, with the latter parameter facilitating the identification of $\text{H}^+$ transport and calculation of the transport ratio between two ions; (3) third, perception of the crystal structure of transporters facilitates the understanding of the $\text{H}^+$ transfer pathway; and (4) mutants with uncoupled $\text{H}^+$ and substrate transport should be observed.
4. Roles of H⁺ Transport in Genetic Plant Improvements and Stress Resistance

4.1. Increasing Yield

H⁺ transport mediated by the above-mentioned proteins involves yield regulation. Examples are listed as follows.

Overexpression of OSA1 in rice significantly increases yield. One reason is that overexpression of this gene significantly enhances the ability of rice to excrete protons, which can not only ensure the homeostasis of cytosolic pH, but also form a stronger proton driving force and enhance the absorption of nutrients by the roots [35].

Overexpression of OsNRT2.3b in rice greatly promotes yield. One reason is that overexpression of this gene leads to phloem sap acidification, which facilitates the transport of P/Fe to the leaves [104].

Overexpression of OsHAK5 in rice notably increases yield. One reason is that overexpression of this gene leads to the alkalization of the extracellular medium, which facilitates the transport of IAA into the cytosol [168].

4.2. Acid Stress Resistance

H⁺ transport mediated by the above-mentioned proteins participates in acid stress resistance. Several lines of evidence are listed below.

Overexpression of an active form of H⁺-ATPase, AHA3-T498D in Arabidopsis, facilitates resistance to acid stress. This phenomenon is attributed to the enhanced excretion of H⁺ from the cytosol, favoring cytosolic pH homeostasis [34].

Overexpression of AtNRT1.1 in Arabidopsis significantly increases the resistance to acid stress. This observation is the result of the enhanced consumption of extracellular H⁺, creating a more favorable rhizosphere pH [177].

5. Conclusions and Prospects

H⁺-ATPases and multiple transporters mediate H⁺ flux across the plasma membrane and are proposed to be essential for cytosolic pH homeostasis in plants. This review focused on seven types of proteins (H⁺-ATPase, NHX, CHX, AMT, NRT, and the KT/HAK/KUP family) that possess direct H⁺ transport activity, concentrating on the following four items: plasma-membrane-located family members, the effect of changes in their expression level on the cytosolic pH, the H⁺ transport pathway, and their functional regulation by the extracellular/cytosolic pH (summarized in Figure 1 and Table 1). Conclusions are drawn as follows. First, each of these seven types of protein is capable of mediating H⁺ flux across the plasma membrane, thus contributing to cytosolic pH homeostasis. However, intra-family members possess distinct H⁺ transport properties, with some members possessing the ability to transport H⁺, whereas other members are unable to transport H⁺. Second, the H⁺ transport activities of each of these seven types of protein are regulated by extracellular and cytosolic pH. However, intra-family members possess distinct pH regulation properties. Third, each of these seven types of protein has different H⁺ transport structural bases, and intra-family members possess different H⁺ transport structural bases.

Table 1. Functional regulation by extracellular and/or cytosolic pH and key residues for H⁺ transport.

| Protein Name | Regulation by pH | Key Residues of H⁺ Transfer Pathway |
|--------------|------------------|------------------------------------|
| H⁺-ATPase family |
| AHA2 | Bell-shaped dependence on cytosolic pH, with maximal transport activity approaching pH 6.6 [47] | D684, N106 and R655 [29,41–43] |
| AHA1&AHA3, NpPMA2 &NpPMA4, and rice H⁺-ATPases | Bell-shaped dependence on cytosolic pH, with maximal transport activity approaching pH 6.0–6.6 [33,40,45–48] |
| AHA7 | Active only when extracellular pH is ≥ 6.0 |

[44]
We believe that the following points necessitate further attention. First, in view of the fact that intra-family members possess distinct H⁺ transport properties and underlying structural bases, using the transport mechanism of a protein to represent the case of the entire family is not suitable. Second, as an accompanying ion that is co-transported by most nutrient uptake transporters, H⁺ receives less attention, leading to the fact the conclusions drawn regarding their H⁺ transport are somewhat imprecise. Subsequent studies regarding H⁺ transport of related proteins should rely on much more solid evidence, which is proposed in Section 3. Third, the matter of how these proteins cooperate to achieve cytosolic pH homeostasis awaits further study [178]. Additionally, except for the seven types of protein, transporters such as H⁺-coupled sucrose transporters (abbreviated as SUT), H⁺-coupled amino acid permease (abbreviated as AAP), and sulfate transporters (abbreviated as SULTR) are also conceived to contribute to the cytosolic pH through direct mediating H⁺ flux across the plasma membrane [8,179–182]. Studies on these proteins, and the coordination of these plasma membrane H⁺ transport proteins, in addition to organelle-located ones, are crucial for the elucidation of the molecular mechanism for cytosolic pH homeostasis. Finally, in addition to maintaining cytoplasmic pH homeostasis, the physiological significance of H⁺ transport mediated by these proteins needs to be further explored, and several examples are provided in Section 4.
Figure 1. Plasma-membrane-located H+ transport proteins. H+-ATPase family functions in mediating H+ efflux from the cytosol to the extracellular space, whereas most members of the NHX, CHX, AMT, NRT, PHT, KT/HAK/KUP, AAP, SULTR, SUT family are responsible for mediating H+ influx from the extracellular space to the cytosol. Notably, several intra-family members of AMT and NRT do not transport H+, indicating that not all plasma membrane transporters possess H+-coupled substrate transport mechanisms. Seven types of H+ transport proteins focused on in this review (H+-ATPase, NHX, CHX, AMT, NRT, PHT, and KT/HAK/KUP) are indicated by black font, whereas other proteins (AAP, SULTR, SUT, etc.) are indicated by the gray font. Specific proteins with experimental evidences (references are indicated by [number]) are presented in the corresponding family. Arrows (↑) indicate the direction of H+ flux. Inability to transport H+ is indicated by special lines (symbols as shown for LeAMT1;1 and AtNRT2.4). Abbreviations: H+-ATPase (H+-pumping ATPase), NHX (Na+/H+ exchanger), CHX (cation/H+ exchanger), AMT (ammonium transporter), NRT (nitrate transporter), PHT (phosphate transporter), KT/HAK/KUP (K+ transporter/high-affinity K+ transporter/K+ uptake permease), SUT (Sucrose transporter), AAP (amino acid permease) and SULTR (sulfate transporter).

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References
1. Schumacher, K. pH in the plant endomembrane system-an import and export business. Curr. Opin. Plant Biol. 2014, 22, 71–76. [CrossRef] [PubMed]
2. Bassil, E.; Blumwald, E. The ins and outs of intracellular ion homeostasis: NHX-type cation/H+ transporters. Curr. Opin. Plant Biol. 2014, 22, 1–6. [CrossRef]
3. Reguera, M.; Bassil, E.; Tajima, H.; Wimmer, M.; Chanoca, A.; Otegui, M.; Paris, N.; Blumwald, E. pH Regulation by NHX-Type Antiporters Is Required for Receptor-Mediated Protein Trafficking to the Vacuole in Arabidopsis. Plant Cell 2015, 27, 1200–1217. [CrossRef]
4. Casey, J.; Grinstein, S.; Orlowski, J. Sensors and regulators of intracellular pH. Nat. Rev. Mol. Cell Biol. 2010, 11, 50–61. [CrossRef] [PubMed]
5. Felle, H.H. pH: Signal and messenger in plant cells. *Plant Biol.* 2001, 3, 577–591. [CrossRef]

6. Pittman, J. Multiple transport pathways for mediating intracellular pH homeostasis: The contribution of H+/ion exchangers. *Front. Plant Sci.* 2012, 3, 11. [CrossRef]

7. Sze, H.; Chanroj, S. Plant endomembrane dynamics: Studies of K+/H+ antiporters provide insights on the effects of pH and ion homeostasis. *Plant Physiol.* 2018, 177, 875–895. [CrossRef]

8. Wegner, L.H.; Shabala, S. Biochemical pH clamp: The forgotten resource in membrane bioenergetics. *New Phytol.* 2020, 225, 37–47. [CrossRef]

9. Kurkdjian, A.; Guern, J. Intracellular pH: Measurement and importance in cell activity. *Annu. Rev. Plant Biol.* 1989, 40, 271–303. [CrossRef]

10. Hochachka, P.W.; Somero, G.N. Biochemical Adaptation. Mechanism and Process. In *Physiological Evolution*; Oxford University Press: Oxford, UK, 2002; pp. 345–351.

11. Niffoles, R.; Rubio, L.; García-Sánchez, M.; Fernández, J.; Bueso, E.; Alejandro, S.; Serrano, R. A dominant-negative form of Arabidopsis AP-3 β-adaptin improves intracellular pH homeostasis. *Plant J.* 2013, 74, 557–568. [CrossRef] [PubMed]

12. Feng, H.; Fan, X.; Miller, A.J.; Xu, G. Plant nitrogen uptake and assimilation: Regulation of cellular pH homeostasis. *J. Exp. Bot.* 2020, 71, 4380–4392. [CrossRef] [PubMed]

13. Davies, D. The fine control of cytosolic pH. *Physiol. Plant.* 1986, 67, 702–706. [CrossRef]

14. Cosse, M.; Seidel, T. Plant proton pumps and cytosolic pH-homeostasis. *Front. Plant Sci.* 2021, 12, 846. [CrossRef] [PubMed]

15. Wegner, L.H.; Li, X.; Zhang, J.; Yu, M.; Shabala, S.; Hao, Z. Biochemical and biophysical pH clamp controlling net H+ efflux across the plasma membrane of plant cells. *New Phytol.* 2021, 230, 408–415. [CrossRef]

16. Isayenko, S.; Dabравolski, S.A.; Pan, T.; Shabala, S. Phylogenetic diversity and physiological roles of plant monovalent cation/H+ antiporters. *Front. Plant Sci.* 2020, 11, 1451. [CrossRef]

17. Falhof, J.; Pedersen, J.T.; Fuglsang, A.T.; Palmgren, M. Plasma Membrane H+-ATPase regulation in the center of plant physiology. *Mol. Plant* 2016, 9, 323–337. [CrossRef] [PubMed]

18. Appelhagen, I.; Nordholt, N.; Seidel, T.; Spelt, K.; Koes, R.; Quattrochio, F.; Sagasser, M.; Weisshaar, B. Transparent testa 13 is a tonoplast P3A-ATPase required for vacuolar deposition of proanthocyanidins in *Arabidopsis thaliana* seeds. *Plant J.* 2015, 82, 840–849. [CrossRef]

19. Li, Y.; Prozenzano, S.; Bleik, M.; Spelt, C.; Appelhagen, I.; Faria, L.M.; Verweij, W.; Schubert, A.; Sagasser, M.; Seidel, T.; et al. Evolution of tonoplast P-ATPase transporters involved in vacuolar acidification. *New Phytol.* 2016, 211, 1092–1107. [CrossRef]

20. Baxter, I.; Tchieu, J.; Sussman, M.; Boutry, M.; Palmgren, M.; Gribskov, M.; Harper, J.; Axelsen, K. Genomic comparison of P-Type ATPase ion pumps in Arabidopsis and Rice. *Plant Physiol.* 2003, 132, 618–628. [CrossRef]

21. Kalampanayil, B.; Wimmers, L. Identification and characterization of a salt-stress-induced plasma membrane H+-ATPase in tomato. *Plant Cell Environ.* 2001, 24, 999–1000. [CrossRef]

22. Santi, S.; Locci, G.; Monte, R.; Pinton, R.; Varanini, Z. Induction of nitrate uptake in maize roots: Expression of a putative high-affinity nitrate transporter and plasma membrane H+-ATPase isoforms. *J. Exp. Bot.* 2003, 54, 1851–1864. [CrossRef] [PubMed]

23. Okumura, M.; Inoue, S.; Takahashi, K.; Ishizaki, K.; Kohchi, T.; Kinoshita, T. Characterization of the Plasma Membrane H+-ATPase in the Liverwort *Marchantia polymorpha*. *Plant Physiol.* 2012, 159, 826–834. [CrossRef] [PubMed]

24. Wdowikowska, A.; Klobus, G. The plasma membrane proton pump gene family in cucumber. *Acta Physiol. Plant.* 2016, 38, 1–14. [CrossRef]

25. Stritzler, M.; García, M.N.; Schlesinger, M.; Corteleezzi, J.J.; Capiati, D. The plasma membrane H+-ATPase gene family in *Solanium tuberosum* L. Role of PHA1 in tuberization. *J. Exp. Bot.* 2017, 68, 4821–4837. [CrossRef] [PubMed]

26. Arango, M.; Gévaudant, F.; Oufattole, M.; Boutry, M. The plasma membrane proton pump ATPase: The significance of gene subfamilies. *Planta* 2002, 216, 355–365. [CrossRef]

27. Xu, Z.; Marowa, P.; Liu, H.; Du, H.; Zhang, C.; Li, Y. Genome-wide identification and analysis of P-Type plasma membrane H+-ATPase sub-genome family in sunflower and the role of HHA4 and HHA11 in the development of salt stress resistance. *Genes* 2020, 11, 361. [CrossRef]

28. Palmgren, M. Plant plasma membrane H+-ATPases: Powerhouses for nutrient uptake. *Annu. Rev. Plant Biol.* 2001, 52, 817–845. [CrossRef]

29. Morth, J.P.; Pedersen, B.P.; Buch-Pedersen, M.; Andersen, J.; Vilsen, B.; Palmgren, M.; Nissen, P. A structural overview of the plasma membrane Na+, K+-ATPase and H+-ATPase ion pumps. *Nat. Rev. Mol. Cell Biol.* 2010, 12, 60–70. [CrossRef]

30. Elmore, J.; Coaker, G. The role of the plasma membrane H+-ATPase in plant-microbe interactions. *Mol. Plant* 2011, 4, 416–427. [CrossRef]

31. Schaller, A.; Ocking, C. Modulation of plasma membrane H+-ATPase activity differentially activates wound and pathogen defense responses in tomato plants. *Plant Cell* 1999, 11, 263–272. [CrossRef]

32. Frick, U.B.; Schaller, A. cDNA microarray analysis of fusicoccin-induced changes in gene expression in tomato plants. *Planta* 2002, 216, 83–94. [CrossRef]

33. Luo, H.; Morsonne, P.; Boutry, M. The two major types of plant plasma membrane H+-ATPases show different enzymatic properties and confer differential pH sensitivity of yeast growth. *Plant Physiol.* 1999, 119, 627–634. [CrossRef]

34. Robertson, W.; Clark, K.; Young, J.C.; Sussman, M. An *Arabidopsis thaliana* plasma membrane proton pump is essential for pollen development. *Genetics* 2004, 168, 1677–1687. [CrossRef]
35. Zhang, M.; Wang, Y.; Chen, X.; Xu, F.; Ding, M.; Ye, W.; Kawaya, Y.; Toda, Y.; Hayashi, Y.; Suzuki, T.; et al. Plasma membrane H+-ATPase overexpression increases rice yield via simultaneous enhancement of nutrient uptake and photosynthesis. *Nat. Commun.* 2021, 12, 735. [CrossRef]

36. Harper, J.; Surowy, T.; Sussman, M. Molecular cloning and sequence of cDNA encoding the plasma membrane proton pump (H+-ATPase) of *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 1989, 86, 1234–1238. [CrossRef]

37. Haruta, M.; Burch, H.L.; Nelson, R.B.; Barrett-Wilt, G.; Kline, K.G.; Mohsin, S.B.; Young, J.C.; Otegui, M.; Sussman, M. Molecular characterization of mutant Arabidopsis plants with reduced plasma membrane proton pump activity. *J. Biol. Chem.* 2010, 285, 17918–17929. [CrossRef] [PubMed]

38. Yamauchi, S.; Takekiyama, A.; Sakamoto, T.; Kurata, T.; Tsutsumi, T.; Kinoshiba, T.; Shimazaki, K. The plasma membrane H+-ATPase AHA1 plays a major role in stomatal opening in response to blue light. *Plant Physiol.* 2016, 171, 2731–2743. [CrossRef] [PubMed]

39. Yuan, W.; Zhang, D.; Song, T.; Xu, F.; Lin, S.; Xu, W.; Li, Q.; Zhu, Y.; Liang, J.; Zhang, J. Arabidopsis plasma membrane H+-ATPase genes AHA2 and AHA7 have distinct and overlapping roles in the modulation of root tip H+ efflux in response to low-phosphorus stress. *J. Exp. Bot.* 2017, 68, 1731–1741. [CrossRef] [PubMed]

40. Hoffmann, R.D.; Portes, M.; Olsen, L.I.; Daminieli, D.S.; Hayashi, M.; Nunes, C.O.; Pedersen, J.T.; Lima, P.T.; Campos, C.; Feijó, J.; et al. Plasma membrane H+-ATPases sustain pollen tube growth and fertilization. *Nat. Commun.* 2020, 11, 1–15. [CrossRef] [PubMed]

41. Buch-Pedersen, M.J.; Pedersen, B.P.; Veierskov, B.; Nissen, P.; Palmgren, M.G. Protons and how they are transported by proton pumps. *Fungi* *Arch.* 2009, 457, 573–579. [CrossRef] [PubMed]

42. Pedersen, B.P.; Buch-Pedersen, M.J.; Morth, J.P.; Palmgren, M.G.; Nissen, P. Crystal structure of the plasma membrane proton pump. *Nature* 2007, 450, 1111–1114. [CrossRef] [PubMed]

43. Buch-Pedersen, M.J.; Palmgren, M.G. Conserved Asp684 in transmembrane segment M6 of the plant plasma membrane P-type proton pump AHA2 is a molecular determinant of proton translocation. *J. Biol. Chem.* 2003, 278, 17845–17851. [CrossRef] [PubMed]

44. Hoffmann, R.D.; Olsen, L.I.; Ezike, C.V.; Pedersen, J.T.; Manstretta, R.; López-Marqués, R.L.; Palmgren, M. Roles of plasma membrane proton ATPases AHA2 and AHA7 in normal growth of roots and root hairs in *Arabidopsis thaliana*. *Physiol. Plant.* 2019, 166, 848–861. [CrossRef] [PubMed]

45. Palme, M.; Christensen, G. Functional comparisons between plant plasma membrane H+-ATPase isoforms expressed in yeast. *J. Biol. Chem.* 1994, 269, 3027–3033. [CrossRef]

46. Regenberg, B.; Villalba, J.M.; Lanfermeijer, F.; Palmgren, M. C-terminal deletion analysis of plant plasma membrane H+-ATPase. *Yeast* as a model system for solute transport across the plant plasma membrane. *Plant Cell* 1995, 7, 1655–1666.

47. Oliviari, C.; Pugliarello, M.; Rasi-Caldogno, F.; Michelis, M.I. Characteristics and regulatory properties of the H+-ATPase in a plasma membrane fraction purified from *Arabidopsis thaliana*. *Bot. Acta* 1993, 106, 13–19. [CrossRef]

48. Zhu, Y.; Di, T.; Xu, G.; Chen, X.; Zeng, H.; Yan, F.; Shen, Q. Adaptation of plasma membrane H+-ATPase of rice roots to low pH as related to ammonium nutrition. *Plant Cell Environ.* 2009, 32, 1428–1440. [CrossRef]

49. Liang, C.J.; Ge, Y.Q.; Su, L.; Bu, J.J. Response of plasma membrane H+-ATPase in rice (*Oryza sativa*) seedlings to simulated acid rain. *Environ. Sci. Pollut. Res. Int.* 2015, 22, 535–545. [CrossRef]

50. Liang, C.; May, L.L. Comparison of plasma membrane H+-ATPase response to acid rain stress between rice and soybean. *Environ. Sci. Pollut. Res. 2019, 27, 6389–6400. [CrossRef]

51. Reid, R.; Field, L.; Pitman, M. Effects of external pH, fuscoicin and butyrate on the cytoplasmic pH in barley root tips measured by 31P-nuclear magnetic resonance spectroscopy. *Planta* 1985, 166, 341–347. [CrossRef]

52. Raven, J.A. Sensing pH? *Plant Cell Environ.* 1990, 13, 721–729. [CrossRef]

53. Bobik, K.; Broutry, M.; Duby, G. Activation of the plasma membrane H+-ATPase by acid stress. *Plant Signal. Behav.* 2010, 5, 681–683. [CrossRef]

54. An, R.; Chen, Q.; Chai, M.; Lu, P.; Su, Z.; Qin, Z.; Chen, J.; Wang, X. AtNHX8, a member of the monovalent cation: Proton antiporter-1 family in *Arabidopsis thaliana*, encodes a putative Li/H antiporter. *Plant J.* 2007, 49, 718–728. [CrossRef]

55. Fu, X.; Lu, Z.; Wei, H.; Zhang, J.; Yang, X.; Wu, A.; Ma, L.; Kang, M.; Lu, J.; Wang, H.; et al. Genome-wide identification and expression analysis of the NHX (Sodium/Hydrogen Antiporter) gene family in cotton. *Front. Genet.* 2020, 11, 964. [CrossRef] [PubMed]

56. Shi, H.; Ishitani, M.; Kim, C.; Zhu, J. The *Arabidopsis thaliana* salt tolerance gene SOS1 encodes a putative Na+/H+ antiporter. *Proc. Natl. Acad. Sci. USA* 2000, 97, 6896–6901. [CrossRef]

57. Aharon, G.S.; Apse, M.P.; Duan, S.; Hua, X.; Blumwald, E. Characterization of a family of vacuolar Na+/H+ antiporters in *Arabidopsis thaliana*. *Plant Soil* 2003, 253, 245–256. [CrossRef]

58. Brett, C.L.; Donowitz, M.; Rao, R. Evolutionary origins of eukaryotic sodium/proton exchangers. *Am. J. Physiol. Cell Physiol.* 2005, 288, 223–239. [CrossRef] [PubMed]

59. Bassil, E.; Tajima, H.; Liang, Y.; Ohto, M.; Ushijima, K.; Nakano, R.; Esumi, T.; Coku, A.; Belmonte, M.; Blumwald, E. The Arabidopsis Na+/H+ antiporters NHX1 and NHX2 control vacuolar pH and K+ homeostasis to regulate growth, flower development, and reproduction. *Plant Cell* 2011, 23, 3482–3497. [CrossRef] [PubMed]

60. Martínez-Atienza, J.; Jiang, X.; García-deblas, B.; Mendoza, I.; Zhu, J.K.; Pardo, J.M.; Quintero, F.J. Conservation of the salt overly sensitive pathway in rice. *Plant Physiol.* 2007, 143, 1001–1012. [CrossRef]

61. Xu, H.; Jiang, X.; Zhan, K.; Cheng, X.; Chen, Pardo, J.M.; Cui, D.Q. Functional characterization of a wheat plasma membrane Na+/H+ antiporter in yeast. *Arch Biochem. Biophys.* 2008, 473, 8–15. [CrossRef]
62. Olias, R.; Eljakaoui, Z.; Li, J.; Marín-Manzano, M.C.; Pardo, J.M.; Belver, A. The plasma membrane Na\(^+\)/H\(^+\) antiporter SOS1 is essential for salt tolerance in tomato and affects the partitioning of Na\(^+\) between plant organs. *Plant Cell Environ.* 2009, 32, 904–916. [CrossRef] [PubMed]

63. Feki, K.; Quintero, F.J.; Khoudi, H.; Leidi, E.; Masmoudi, K.; Pardo, J.M.; Brini, F. A constitutively active form of a durum wheat Na\(^+\)/H\(^+\) antiporter SOS1 confers high salt tolerance to transgenic Arabidopsis. *Plant Cell Rep.* 2013, 33, 277–288. [CrossRef]

64. Qiu, Q.S.; Barkla, B.J.; Vera-Estrella, R.; Zhu, J.K.; Schumaker, K.S. Na\(^+\)/H\(^+\) exchange activity in the plasma membrane of Arabidopsis. *Plant Physiol.* 2003, 132, 1041–1052. [CrossRef] [PubMed]

65. Munns, R. Comparative physiology of salt and water stress. *Plant Cell Environ.* 2002, 25, 239. [CrossRef]

66. Dutta, D.; Esmaill, M.; Overduin, M.; Fliegel, L. Expression and detergent free purification and reconstitution of the plant plasma membrane Na\(^+\)/H\(^+\) antiporter SOS1 overexpressed in *Pichia pastoris*. *Biochim. Biophys. Acta Biomembr.* 2019, 1862, 183111. [CrossRef]

67. Hall, D.; Evans, A.R.; Newbury, H.J.; Pritchard, J. Functional analysis of CHX21: A putative sodium transporter in Arabidopsis. *Plant Physiol.* 2005, 139, 161–170. [CrossRef]

68. Dragwidge, J.; Scholl, S.; Schumacher, K.; Gendall, A. NHX-type Na\(^+\)/(K\(^+\))/H\(^+\) antiporters are required for TGN/EE trafficking and endosomal ion homeostasis in *Arabidopsis thaliana*. *J. Cell. Sci.* 2019, 132, jcs226472. [CrossRef]

69. Mottaleb, S.A.; Rodríguez-Salazar, J.; Albert, A. Structural insights on the plant salt-overly-sensitive 1 (SOS1) Na\(^+\)/H\(^+\) antiporter. *J. Mol. Biol.* 2012, 424, 283–294. [CrossRef]

70. Bassil, E.; Coku, A.; Blumwald, E. Cellular ion homeostasis: Emerging roles of intracellular NHX Na\(^+\)/H\(^+\) antiporters in plant growth and development. *J. Exp. Bot.* 2012, 63, 5727–5740. [CrossRef] [PubMed]

71. Shabala, L.; Cuin, T.A.; Newman, I.A.; Shabala, S. Salinity-induced ion flux patterns from the excised roots of Arabidopsis sos mutants. *Plant Biol.* 2005, 222, 1041–1050. [CrossRef] [PubMed]

72. Guo, K.; Babourina, O.; Rengel, Z. Na\(^+\)/H\(^+\) antiporter activity of the SOS1 gene: Lifetime imaging analysis and electrophysiological studies on Arabidopsis seedlings. *Physiol. Plant.* 2009, 137, 155–165. [CrossRef] [PubMed]

73. Núñez-Ramírez, R.; Sánchez-Barrena, M.; Villalta, I.; Vega, J.; Pardo, J.M.; Quintero, F.J.; Martínez-Salazar, J.; Albert, A. Structural insights on the plant salt-overly-sensitive 1 (SOS1) Na\(^+\)/H\(^+\) antiporter. *J. Mol. Biol.* 2012, 424, 283–294. [CrossRef] [PubMed]

74. Zhao, J.; Li, P.; Motes, C.M.; Park, S.; Hirschi, K.D. CHX14 is a plasma membrane K\(^+\)/H\(^+\) antiporter. *Plant Physiol.* 2008, 148, 796–807. [CrossRef]

75. Zhao, J.; Li, P.; Motes, C.M.; Park, S.; Hirschi, K.D. CHX14 is a plasma membrane K\(^+\)/H\(^+\) antiporter that regulates K\(^+\) redistribution in *Arabidopsis thaliana*. *Plant Cell Environ.* 2015, 38, 2223–2238. [CrossRef] [PubMed]

76. Chanroj, S.; Padmanaban, S.; Czerny, D.D.; Jauh, G.Y.; Sze, H. K\(^+\) transporter AtCHX17 with its hydrophilic C tail localizes to membranes of the secretory/endocytic system: Role in reproduction and seed set. *Mol. Plant* 2013, 6, 1226–1246. [CrossRef]

77. Padmanaban, S.; Czerny, D.D.; Levin, K.A.; Leydon, A.R.; Su, R.T.; Mauge, T.K.; Zou, Y.; Chanroj, S.; Cheung, A.Y.; Johnson, M.A. Transports involved in plasma membrane Na\(^+\)/H\(^+\) antiporter CHX17 and its homologs are endomembrane K\(^+\) transporters with roles in protein sorting. *J. Biol. Chem.* 2011, 286, 33931–33941. [CrossRef] [PubMed]

78. Hao, D.; Zhou, J.; Yang, S.; Qi, W.; Yang, K.; Su, Y. Function and regulation of ammonium transporters in plants. *Int. J. Mol. Sci.* 2020, 21, 3557. [CrossRef]

79. Segard, R.; Alsterford, M.; MacAulay, N.; Zeuthen, T. Ammonium ion transport by the AMT/Rh homolog TaAMT1;1 is stimulated by acidic pH. *Pflüg. Arch.-Eur. J. Physiol.* 2009, 458, 733–743. [CrossRef]

80. Neuhäuser, B.; Ludewig, U. Uncoupling of ionic currents from substrate transport in the plant ammonium transporter AtAMT1;2. *J. Biol. Chem.* 2014, 289, 11650–11655. [CrossRef]
89. Ortiz-Ramirez, C.; Mora, S.I.; Trejo, J.M.; Pantoja, O. PvAMT1;1, a highly selective ammonium transporter that functions as H+/NH4+ symporter. *J. Biol. Chem.* 2011, 286, 31113–31122. [CrossRef]

90. Neuhäuser, B.; Dynowska, M.; Mayer, M.G.; Ludewig, U. Regulation of NH4+ transport by essential cross talk between AMT monomers through the carboxyl tails. *Plant Physiol.* 2007, 143, 1651–1659. [CrossRef]

91. Li, T.; Liao, K.; Xu, X.; Gao, Y.; Wang, Z.; Zhu, X.; Jia, B.; Xuan, Y. Wheat ammonium transporter (AMT) gene family: Diversity and possible role in host–pathogen interaction with stem rust. *Front. Plant Sci.* 2017, 8, 1637. [CrossRef]

92. Mayer, M.; Dynowska, M.; Ludewig, U. Ammonium ion transport by the AMT/Rh homologue LeAMT1;1. *Biochem. J.* 2006, 396, 431–437. [CrossRef] [PubMed]

93. Wang, Y.; Hsu, P.; Tsay, Y. Uptake, allocation and signaling of nitrate. *Trends Plant Sci.* 2012, 17, 458–467. [CrossRef] [PubMed]

94. Krapp, A.; David, L.; Chardin, C.; Girin, T.; Marmagne, A.; Leprince, A.; Chaillou, S.; Ferrario-Möry, S.; Meyer, C.; Daniel-Vedele, F. Nitrate transport and signalling in Arabidopsis. *J. Exp. Bot.* 2014, 65, 789–798. [CrossRef] [PubMed]

95. Fan, X.; Naz, M.; Fan, X.; Xuan, W.; Miller, A.; Xu, G. Plant nitrate transporters: From gene function to application. *J. Exp. Bot.* 2017, 68, 2463–2475. [CrossRef] [PubMed]

96. Wang, Y.; Cheng, Y.; Chen, K.; Tsay, Y. Nitrate transport, signaling, and use efficiency. *Annu. Rev. Plant Biol.* 2018, 69, 85–122. [CrossRef] [PubMed]

97. Zhou, J.; Theodoulou, F.; Muldin, I.; Ingemarsson, B.; Miller, A.J. Cloning and functional characterization of a *Brassica napus* transporter that is able to transport nitrate and histidine. *J. Biol. Chem.* 1998, 273, 12017–12023. [CrossRef]

98. Tsay, Y.F.; Schroeder, J.I.; Feldmann, K.A.; Crawford, N.M. The herbicide sensitivity gene CHL1 of Arabidopsis encodes a nitrate-inducible nitrate transporter. *Cell* 1993, 72, 705–713. [CrossRef]

99. Ho, C.H.; Lin, S.H.; Hu, H.C.; Frommer, W. Fluorescent sensors for activity and regulation of the nitrate transceptor CHL1/NRT1.1 and oligopeptide transporter NRT1.1. *Plant Physiol.* 2010, 151, 1513–1519. [CrossRef] [PubMed]

100. Chen, J.; Bankston, J.R.; Payandeh, J.; Hinds, T.R.; Zagotta, W.N.; Zheng, N. Crystal structure of the plant dual-affinity nitrate transporter NRT1.1. *Nature* 2014, 510, 73–77. [CrossRef] [PubMed]

101. Ho, C.; Frommer, W. Fluorescent sensors for activity and regulation of the nitrate transceptor CHL1/NRT1.1 and oligopeptide transporters. *eLife* 2014, 3, e01917. [CrossRef] [PubMed]

102. Jorgensen, M.E.; Olsen, C.E.; Geiger, D.; Mirza, O.; Halkier, B.A.; NourEldin, H.H. A functional EXXEK motif is essential for proton coupling and active glucosinolate transport by NPF2.11. *Plant Cell Physiol.* 2015, 56, 2340–2350. [CrossRef] [PubMed]

103. Parker, J.L.; Li, C.; Brinth, A.; Wang, Z.; Vogeley, L.; Solcan, N.; Ledderboge-Vucinic, G.; Swanson, J.; Caffrey, M.; Voth, G.; et al. Proton movement and coupling in the P0T family of peptide transporters. *Proc. Natl. Acad. Sci. USA* 2017, 114, 13182–13187. [CrossRef] [PubMed]

104. Parker, J.L.; Newstead, S. Molecular basis of nitrate uptake by the plant nitrate transporter NRT1.1. *Nature* 2014, 507, 68–72. [CrossRef] [PubMed]

105. Liu, K.; Huang, C.; Tsay, Y. CHL1 is a dual-affinity nitrate transporter of Arabidopsis involved in multiple phases of nitrate uptake. *Plant Cell* 1999, 11, 865–874. [CrossRef]

106. Mudge, S.R.; Rae, A.; Diatloff, E.; Smith, F. Expression analysis suggests novel roles for members of the PtH1 family of phosphate transporters in Arabidopsis. *Plant J.* 2002, 31, 341–353. [CrossRef] [PubMed]

107. Nussaume, L.; Kanno, S.; Javot, H.; Marin, E.; Pochnon, N.; Ayadi, A.; Nakaniishi, T.; Thibaud, M. Phosphate import in plants: Focus on the PHT1 transporters. *Front. Plant Sci.* 2011, 2, 83. [CrossRef]
118. Liu, F.; Xu, Y.J.; Jiang, H.H.; Jiang, C.S.; Du, Y.B.; Gong, C.; Wang, W.; Zhu, S.W.; Han, G.M.; Cheng, B.J. Systematic identification, evolution and expression analysis of the Zea mays PHT1 gene family reveals several new members involved in root colonization by arbuscular mycorrhizal fungi. *Int. J. Mol. Sci.* 2016, 17, 930. [CrossRef] [PubMed]

119. Wang, D.; Lv, S.; Jiang, P.; Li, Y. Roles, regulation, and agricultural application of plant phosphate transporters. *Front. Plant Sci.* 2017, 8, 817. [CrossRef]

120. Wang, F.; Deng, M.J.; Xu, J.M.; Zhu, X.L.; Mao, C.Z. Molecular mechanisms of phosphate transport and signaling in higher plants. *Semin. Cell Dev. Biol.* 2018, 74, 114–122. [CrossRef]

121. Roch, G.V.; Maharajan, T.; Ceasar, S.A.; Ignacimuthu, S. The Role of PHT1 family transporters in the acquisition and redistribution of phosphorus in plants. *Crit. Rev. Plant Sci.* 2019, 38, 171–198. [CrossRef]

122. Shin, H.; Shin, H.; Dewbre, G.; Harrison, M.J. Phosphate transport in Arabidopsis: Pht1;1 and Pht1;4 play a major role in phosphate acquisition from both low- and high-phosphate environments. *Plant J.* 2004, 39, 629–642. [CrossRef] [PubMed]

123. Bayle, V.; Arrighi, J.; Creff, A.; Nespolous, C.; Vialaret, J.; Rossignol, M.; González, E.; Paz-Ares, J.; Nuusauame, L. *Arabidopsis thaliana* high-affinity phosphate transporters exhibit multiple levels of posttranslational regulation. *Plant Cell* 2011, 23, 1523–1535.

124. Remy, E.; Cabrillo, T.R.; Battista, R.A.; Teixeira, M.C.; Sá-Correia, I.; Duque, P. The Pht1;9 and Pht1;8 transporters mediate inorganic phosphate acquisition by the *Arabidopsis thaliana* root during phosphorus starvation. *New Phytol.* 2012, 195, 356–371. [CrossRef] [PubMed]

125. Chang, M.; Gu, M.; Xia, Y.; Dai, X.; Dai, C.R.; Zhang, J.; Wang, S.; Qu, H.; Yamaji, N.; Ma, J.F.; et al. OsPHT1;3 mediates uptake, translocation, and remobilization of phosphate under extremely low phosphate regimes. *Plant Physiol.* 2018, 179, 656–670. [CrossRef] [PubMed]

126. Ye, Y.; Yuan, J.; Chang, X.; Yang, M.; Zhang, L.; Lu, K.; Lian, X. The phosphate transporter gene OsPht1;4 is involved in phosphate homeostasis in rice. *Plos ONE* 2015, 10, e0126186.

127. Jia, H.; Ren, H.; Gu, M.; Zhao, J.; Sun, S.; Zhang, X.; Chen, J.; Wu, P.; Xu, G. The phosphate transporter gene OsPht1;8 is involved in phosphate homeostasis in rice. *Plant Physiol.* 2011, 156, 1164–1175. [CrossRef]

128. Preuss, C.; Huang, C.; Tyerman, S. Proton-coupled high-affinity phosphate transport revealed from heterologous characterization in Xenopus of barley-root plasma membrane transporter, HvPHT1;1. *Plant Cell Environ.* 2011, 34, 681–689. [CrossRef]

129. Preuss, C.; Huang, C.; Gillham, M.; Tyerman, S. Channel-like characteristics of the low-affinity barley phosphate transporter phl1;6 when expressed in Xenopus oocytes. *Plant Physiol.* 2010, 152, 1431–1441. [CrossRef]

130. Rausch, C.; Bucher, M. Molecular mechanisms of phosphate transport in plants. *Planta* 2002, 216, 23–37. [CrossRef]

131. Młodzińska, E.; Zboińska, M. Phosphate uptake and allocation—A closer look at *Arabidopsis thaliana* L. and *Oryza sativa* L. *Front. Plant Sci.* 2016, 7, 1198. [CrossRef]

132. Ullrich, C.I.; Novacky, A.J. Extra- and intracellular pH and membrane potential changes induced by K⁺, Cl−, H₂PO₄⁻, and NO₃⁻ uptake and fusicoccin in root hairs of *Limmnobium stoloniferum*. *Plant Physiol.* 1990, 94, 1561–1567. [CrossRef]

133. Mimura, T.; Yin, Z.H.; Wirth, E.; Dietz, K.J. Phosphate transport and apoplastic phosphate homeostasis in barley leaves. *Plant Cell Physiol.* 1992, 33, 563–568.

134. Sakano, K.; Yazaki, Y.; Mimura, T. Cytoplasmic acidification induced by inorganic phosphate uptake in suspension cultured *Catharanthus roseus* cells—Measurement with fluorescent pH indicator and P-31 nuclear-magnetic-resonance. *Plant Physiol.* 1990, 99, 672–680. [CrossRef] [PubMed]

135. Pedersen, B.P.; Kumar, H.; Waigt, A.B.; Risenmay, A.J.; Roe-Zurz, Z.; Chau, B.H.; Schlessinger, A.; Bonomi, M.; Harries, W.; Sali, A.; et al. Crystal structure of a eukaryotic phosphate transporter. *Nature* 2013, 496, 533–536. [CrossRef]

136. Liu, Y.; Li, C.; Gupta, M.; Verma, N.; Johri, A.; Stroud, R.; Voth, G. Key computational findings reveal proton transfer as driving the functional cycle in the phosphate transporter PiPT. *Proc. Natl. Acad. Sci. USA* 2021, 118, e2101932118. [CrossRef]

137. Liao, Y.; Li, J.; Pan, R.; Chiou, T. Structure–function analysis reveals amino acid residues of Arabidopsis phosphate transporter AtPHT1;1 crucial for its activity. *Front. Plant Sci.* 2019, 10, 1158. [CrossRef] [PubMed]

138. Pedersen, B.P.; Kumar, H.; Waigt, A.B.; Risenmay, A.J.; Roe-Zurz, Z.; Chau, B.H.; Schlessinger, A.; Bonomi, M.; Harries, W.; Sali, A.; et al. Crystal structure of a eukaryotic phosphate transporter. *Nature* 2013, 496, 533–536. [CrossRef]

139. Liu, Y.; Li, C.; Gupta, M.; Verma, N.; Johri, A.; Stroud, R.; Voth, G. Key computational findings reveal proton transfer as driving the functional cycle in the phosphate transporter PiPT. *Proc. Natl. Acad. Sci. USA* 2021, 118, e2101932118. [CrossRef]

140. Liu, Y.; Li, C.; Gupta, M.; Verma, N.; Johri, A.; Stroud, R.; Voth, G. Key computational findings reveal proton transfer as driving the functional cycle in the phosphate transporter PiPT. *Proc. Natl. Acad. Sci. USA* 2021, 118, e2101932118. [CrossRef]

141. Ma, B.; Zhang, L.; Gao, Q.; Wang, J.; Li, X.; Wang, H.; Liu, Y.; Lin, H.; Liu, J.; Wang, X.; et al. A plasma membrane transporter coordinates phosphate reallocation and grain filling in cereals. *Nat. Genet.* 2021, 53, 906–915. [CrossRef]

142. Mäser, P.; Thomine, S.; Schroeder, J.; Ward, J.; Hirschi, K.; Sze, H.; Talke, I.; Antmann, A.; Maathuis, F.; Sanders, D.; et al. Phylogenetic relationships within cation transporter families of Arabidopsis. *Plant Physiol.* 2001, 126, 1646–1667. [CrossRef] [PubMed]

143. Véry, A.; Sentenc, H. Molecular mechanisms and regulation of K⁺ transport in higher plants. *Annu. Rev. Plant Biol.* 2003, 54, 575–603. [CrossRef] [PubMed]

144. Véry, A.; Nieves-Cordon, M.; Daly, M.; Khan, I.; Fizames, C.; Sentenc, H. Molecular biology of K⁺ transport across the plant cell membrane: What do we learn from comparison between plant species? *J. Plant Physiol.* 2014, 174, 748–769. [CrossRef] [PubMed]
145. Santa-María, G.; Oliferuk, S.; Moriconi, J.I. KT-HAK-KUP transporters in major terrestrial photosynthetic organisms: A twenty years tale. *J. Plant Physiol.* 2018, 226, 77–90. [CrossRef]

146. Gupta, M.; Qiu, X.; Wang, L.; Xie, W.; Zhang, C.; Xiong, L.; Zhang, Q. KT/HAK/KUP potassium transporters gene family and their whole-life cycle expression profile in rice (*Oryza sativa*). *Mol. Genet. Genom.* 2008, 280, 437–452. [CrossRef] [PubMed]

147. Yang, T.; Zhang, S.; Hu, Y.; Wu, F.; Hu, Q.; Chen, G.; Cai, J.; Wu, T.; Moran, N.; Yu, L. The role of a potassium transporter OsHAK5 alters rice architecture via ATP-dependent transmembrane auxin fluxes. *Plant Commun.* 2020, 1, 100052. [CrossRef]

148. Qi, Z.; Hampton, C.R.; Shin, R.; Barkla, B.J.; White, P.J.; Schachtman, D.P. The high affinity K+ transporter AtHAK5 plays a physiological role in planta at very low K+ concentrations and provides a caesium uptake pathway in Arabidopsis. *J. Exp. Bot.* 2008, 59, 595–607. [CrossRef] [PubMed]

149. Rajappa, S.; Krishnamurthy, P.; Kumar, P.P. Regulation of AtKUP2 expression by bHLH and WRKY transcription factors helps to confer increased salt tolerance to *Arabidopsis thaliana* plants. *Front. Plant Sci.* 2020, 11, 1311. [CrossRef] [PubMed]

150. Rigas, S.; Ditengou, F.A.; Ljung, K.; Daras, G.; Tietz, O.; Palme, K.; Hatzopoulos, P. Root gravitropism and root hair development constitute coupled developmental responses regulated by auxin homeostasis in the Arabidopsis root apex. *New Phytol.* 2013, 197, 1130–1141. [CrossRef]

151. Osaka, Y.; Arinaga, N.; Umezawa, T.; Katsura, S.; Nagamachi, K.; Tanaka, H.; Ohiraki, H.; Yamada, K.; Seo, S.-U.; Abo, M. Osmotic stress responses and plant growth controlled by potassium transporters in Arabidopsis. *Plant Cell* 2013, 25, 609–624. [CrossRef] [PubMed]

152. Han, M.; Wu, W.; Wu, W.-H.; Wang, Y. Potassium transporter KUP7 is involved in K+ acquisition and translocation in Arabidopsis root under K+-limited conditions. *Mol. Plant* 2016, 9, 437–446. [CrossRef]

153. Chen, G.; Hu, Q.; Luo, L.; Yang, T.; Zhang, S.; Hu, Y.; Yu, L.; Xu, G. Rice potassium transporter OsHAK1 is essential for maintaining potassium-mediated growth and functions in salt tolerance over low and high potassium concentration ranges. *Plant Cell Environ.* 2015, 38, 2747–2765. [CrossRef] [PubMed]

154. Liu, L.; Zheng, C.; Kuang, B.; Wei, L.; Yan, L.; Wang, T. Receptor-like kinase RUPO interacts with potassium transporters to regulate pollen tube growth and integrity in rice. *PLoS Genet.* 2016, 12, e1006085. [CrossRef]

155. Horie, T.; Sugawara, M.; Okada, T.; Taira, K.; Kaathien-Nakayama, P.; Katsuhara, M.; Shinmyo, A.; Nakayama, H. Rice sodium-insensitive potassium transporter, OsHAK5, confers increased salt tolerance in tobacco BY2 cells. *J. Biosci. Bioeng.* 2011, 111, 346–356. [CrossRef] [PubMed]

156. Yang, T.; Zhang, S.; Hu, Y.; Wu, F.; Hu, Q.; Chen, G.; Cai, J.; Wu, T.; Moran, N.; Yu, L. The role of a potassium transporter OsHAK5 in potassium acquisition and transport from roots to shoots in rice at low potassium supply levels. *Plant Physiol.* 2014, 166, 945–959. [CrossRef] [PubMed]

157. Shen, Y.; Shen, L.; Shen, Z.; Jing, W.; Ge, H.; Zhao, J.; Zhang, W. The potassium transporter OsHAK21 functions in the maintenance of ion homeostasis and tolerance to salt stress in rice: OsHAK21 involvement in responses to salt stress. *Plant Cell Environ.* 2015, 38, 2766–2779. [CrossRef]

158. Maathuis, F.J.; Sanders, D. Mechanism of high-affinity potassium uptake in roots of *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 1994, 91, 9272–9276. [CrossRef] [PubMed]

159. Walker, D.J.; Leigh, R.A.; Miller, A.J. Potassium homeostasis in vacuolate plant cells. *Proc. Natl. Acad. Sci. USA* 1996, 93, 10510–10514. [CrossRef] [PubMed]

160. Nieves-Cordones, M.; Alemán, F.; Martínez, V.; Rubio, F. K+ uptake in plant roots: The systems involved, their regulation and parallels in other organisms. *J. Plant Physiol.* 2014, 171, 688–695. [CrossRef]

161. Rubio, F.; Arévalo, L.; Caballero, F.; Botella, M.A.; Rubio, J.S.; García-Sánchez, F.; Martínez, V. Systems involved in K+ uptake from diluted solutions in pepper plants as revealed by the use of specific inhibitors. *J. Plant Physiol.* 2010, 167, 1494–1499. [CrossRef]

162. Alemán, F.; Caballero, F.; Ródenas, R.; Rivero, R.M.; Martínez, V.; Rubio, F. The F130S point mutation in the Arabidopsis high-affinity K+ transporter AtHAK5 increases K+ over Na+ and Cs+ selectivity and confers Na+ and Cs+ tolerance to yeast under heterologous expression. *Front. Plant Sci.* 2014, 5, 430. [CrossRef]

163. Rubio, F.; Nieves-Cordones, M.; Alemán, F.; Martínez, V. Relative contribution of AtHAK5 and AtAKT1 to K+ uptake in the high-affinity range of concentrations. *Physiol. Plant* 2008, 134, 598–608. [CrossRef] [PubMed]

164. Haro, R.; Sainz, L.; Rubio, F.; Rodríguez-Navarro, A. Cloning of two genes encoding potassium transporters in Neurospora crassa and expression of the corresponding cDNAs in *Saccharomyces cerevisiae*. *Mol. Microbiol.* 1999, 31, 511–520. [CrossRef] [PubMed]

165. Rivetta, A.; Allen, K.E.; Slayman, C.W.; Slayman, C.L. Coordination of K transporters in Neurospora: TRK1 is scarce and constitutive, while HAK1 is abundant and highly regulated. *Euk. Cell* 2013, 12, 684–696. [CrossRef] [PubMed]

166. Scherzer, S.; Böhm, J.; Krol, E.; Shabala, L.; Kreuzer, I.; Larisch, C.; Bemm, F.; Al-Rasheid, K.A.; Shabala, S.; Rennenberg, H. Calcium sensor kinase activates potassium uptake systems in gland cells of *Venus flytraps*. *Plant Physiol. Plant Commun.* 2020, 11, 7309–7314. [CrossRef] [PubMed]

167. Tascón, I.; Sousa, J.S.; Corey, R.; Mills, D.; Grivatz, D.; Aumüller, N.; Mikušević, V.; Stansfeld, P.; Vonck, J.; Hänel, I. Structural basis of proton-coupled potassium transport in the KUP family. *Nat. Commun.* 2020, 11, 1–10. [CrossRef]

168. Yang, T.; Feng, H.; Zhang, S.; Xiao, H.; Hu, Q.; Chen, G.; Xuan, W.; Moran, N.; Murphy, A.S.; Yu, L.; et al. The potassium transporter OsHAK5 alters rice architecture via ATP-dependent transmembrane auxin fluxes. *Plant Commun.* 2020, 1, 100052. [CrossRef]
169. Ródenas, R.; Ragel, P.; Nieves-Cordones, M.; Martínez-Martínez, A.; Amo, J.; Lara, A.; Martínez, V.; Quintero, F.J.; Pardo, J.M.; Rubio, F. Insights into the mechanisms of transport and regulation of the Arabidopsis high-affinity K⁺ transporter HAK5. *Plant Physiol.* 2021, 185, 1860–1874. [CrossRef]

170. Senn, M.E.; Rubio, F.; Bánuelos, M.A.; Rodríguez-Navarro, A. Comparative functional features of plant potassium HvHAK1 and HvHAK2 transporters. *J. Biol. Chem.* 2001, 276, 44563–44569. [CrossRef]

171. García-deblas, B.; Benito, B.; Rodríguez-Navarro, A. Molecular cloning and functional expression in bacteria of the potassium transporters CnHAK1 and CnHAK2 of the seagrass *Cymodocea nodosa*. *Plant Mol. Biol.* 2002, 50, 623–633. [CrossRef]

172. Wang, M.Y.; Glass, A.; Shaff, J.E.; Kochian, L.V. Ammonium Uptake by Rice Roots (III. Electrophysiology). *Plant Physiol.* 1994, 103, 1259–11267. [CrossRef]

173. Sakano, K. Proton/Phosphate Stoichiometry in uptake of inorganic phosphate by cultured cells of *Catharanthus roseus* (L.) G. Don. *Plant Physiol.* 1990, 93, 479–483. [CrossRef]

174. Wang, Y.; Blatt, M.R.; Chen, Z.-H. Ion Transport at the Plant Plasma Membrane; John Wiley & Sons, Ltd.: Chichester, UK, 2018.

175. Neuhäuser, B.; Dynowski, M.; Ludewig, U. Channel-like NH₃ flux by ammonium transporter AtAMT2. *FEBS Lett.* 2009, 583, 2833–2838. [CrossRef]

176. Ludewig, U.; Von Wirén, N.; Frommer, W.B. Uniport of NH₄⁺ by the root hair plasma membrane ammonium transporter LeAMT1;1. *J. Biol. Chem.* 2002, 277, 13548–13555. [CrossRef] [PubMed]

177. Ye, J.Y.; Tian, W.H.; Zhou, M.; Zhu, Q.Y.; Du, W.X.; Zhu, Y.X.; Liu, X.X.; Lin, X.Y.; Zheng, S.J.; Jin, C.W. STOP1 activates NRT1.1-mediated nitrate uptake to create a favorable rhizospheric pH for plant adaptation to acidity. *Plant Cell* 2021, 226, koab226. [CrossRef] [PubMed]

178. Miranda, R.; Mesquita, R.O.; Costa, J.H.; Alvarez-Pizarro, J.; Prisco, J.T.; Gomes-Filho, E. Integrative control between proton pumps and sos1 antiporters in roots is crucial for maintaining low Na⁺ accumulation and salt tolerance in ammonium-supplied *Sorghum bicolor*. *Plant Cell Physiol.* 2017, 8, 522–536. [CrossRef] [PubMed]

179. Tegeder, M.; Masclaux-Daubresse, C. Source and sink mechanisms of nitrogen transport and use. *New Phytol.* 2018, 217, 35–53. [CrossRef]

180. Tegeder, M.; Hammes, U.Z. The way out and in: Phloem loading and unloading of amino acids. *Curr. Opin. Plant Biol.* 2018, 43, 16–21. [CrossRef]

181. Wang, L.; Chen, K.; Zhou, M. Structure and function of an *Arabidopsis thaliana* sulfate transporter. *Nat. Commun.* 2021, 12, 1–8.

182. Santiago, J.P.; Ward, J.M.; Sharkey, T.D. Phaseolus vulgaris SUT1.1 is a high affinity sucrose-proton co-transporter. *Plant Direct* 2020, 4, e00260. [CrossRef]