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Trends in Plant Science

Review

Plasma membrane H^+-ATPases in mineral nutrition and crop improvement

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Plasma membrane H^+-ATPases (PMAs) pump H^+ out of the cytoplasm by consuming ATP to generate a membrane potential and proton motive force for the transmembrane transport of nutrients into and out of plant cells. PMAs are involved in nutrient acquisition by regulating root growth, nutrient uptake, and translocation, as well as the establishment of symbiosis with arbuscular mycorrhizas. Under nutrient stresses, PMAs are activated to pump more H^+ and promote organic anion excretion, thus improving nutrient availability in the rhizosphere. Herein we review recent progress in the physiological functions and the underlying molecular mechanisms of PMAs in the efficient acquisition and utilization of various nutrients in plants. We also discuss perspectives for the application of PMAs in improving crop production and quality.

Highlights

Plasma membrane H^+-ATPases (PMAs) provide an H^+ electrochemical gradient to establish a proton motive force and membrane potential for the transport of various nutrients across the plasma membrane.

PMAs are regulated at multiple levels, and genetic modulation of PMA expression could enhance nutrient acquisition and use efficiency in plants.

PMAs are involved in the coordination of the uptake and assimilation of both nitrate and ammonium.

PMAs are involved in nutrient utilization by regulating root growth, facilitating organic anion exudation, and promoting the acquisition of nutrients through H^+ extrusion-mediated apoplast and rhizosphere acidification.

PMAs play essential roles in mycorrhizal symbioses in plant roots for nutrient acquisition.

PMAs in plants

An important type of transporter in plants, proton (H^+) pumps consume the energy stored by ATP or pyrophosphate to transport H^+ across biological membranes against the concentration gradient, thus providing a **proton motive force** (see Glossary) and **membrane potential** for the transmembrane transport of nutrient ions, sugars, amino acids, and organic anions. **Proton pumps** in plants are generally divided into the **plasma membrane (PM) H^+-ATPase (PMA)**, the vacuolar H^+-ATPase (VHA), and the H^+-pyrophosphatase (H^+-PPase) [1,2]. Plant VHA is a kind of multi-subunit pump which is generally composed of 13 protein subunits that are localized mainly in the vacuolar membrane and endomembrane compartments within the secretory pathway [1,3]. H^+-PPases are located mainly in the tonoplast, but some of them are also located in the Golgi apparatus and the PM [4,5]. Arabidopsis (*Arabidopsis thaliana*) *AHA1* and *AHA3* were the first PMA genes identified in plants [6,7]. Most PMA proteins are located on the PM, but a few PMAs – such as arabidopsis AHA10 and its homolog in petunia (*Petunia hybrida*), PH5 – are located in the tonoplast [8–11].

PMA belongs to the P3A subfamily of P-type ATPase superfamily, which forms a phosphorylated intermediate during the hydrolysis of ATP, and returns to its original conformation after H^+ extrusion to complete a cycle. PMA is structurally similar to other P-type ATPases, such as Ca^{2+}-ATPases, Cu^{2+}/Zn^{2+}-ATPases and Na^+/K^+-ATPases [12]. PMAs are a multigene family; for example, there are 11, 10, 9, 8, and 24 gene members in arabidopsis, rice (*Oryza sativa*), tobacco (*Nicotiana tabacum*), tomato (*Solanum lycopersicum*), and soybean (*Glycine max*), respectively [13,14]. The PMA gene family in plants can be divided into ten subclasses, and PMA genes in vascular plants are distributed mainly into five subclasses (I–V). Unlike VHA, which has multiple subunits, PMA is composed of a single polypeptide of about 100 kDa. PMA generally contains ten transmembrane domains. The rest of the protein is basically located in the cytoplasm, including the N terminal domain and C terminal self-inhibitory domain [15] (Figure 1A). The phosphorylation domain located between the fourth and fifth transmembrane domains is mainly responsible for

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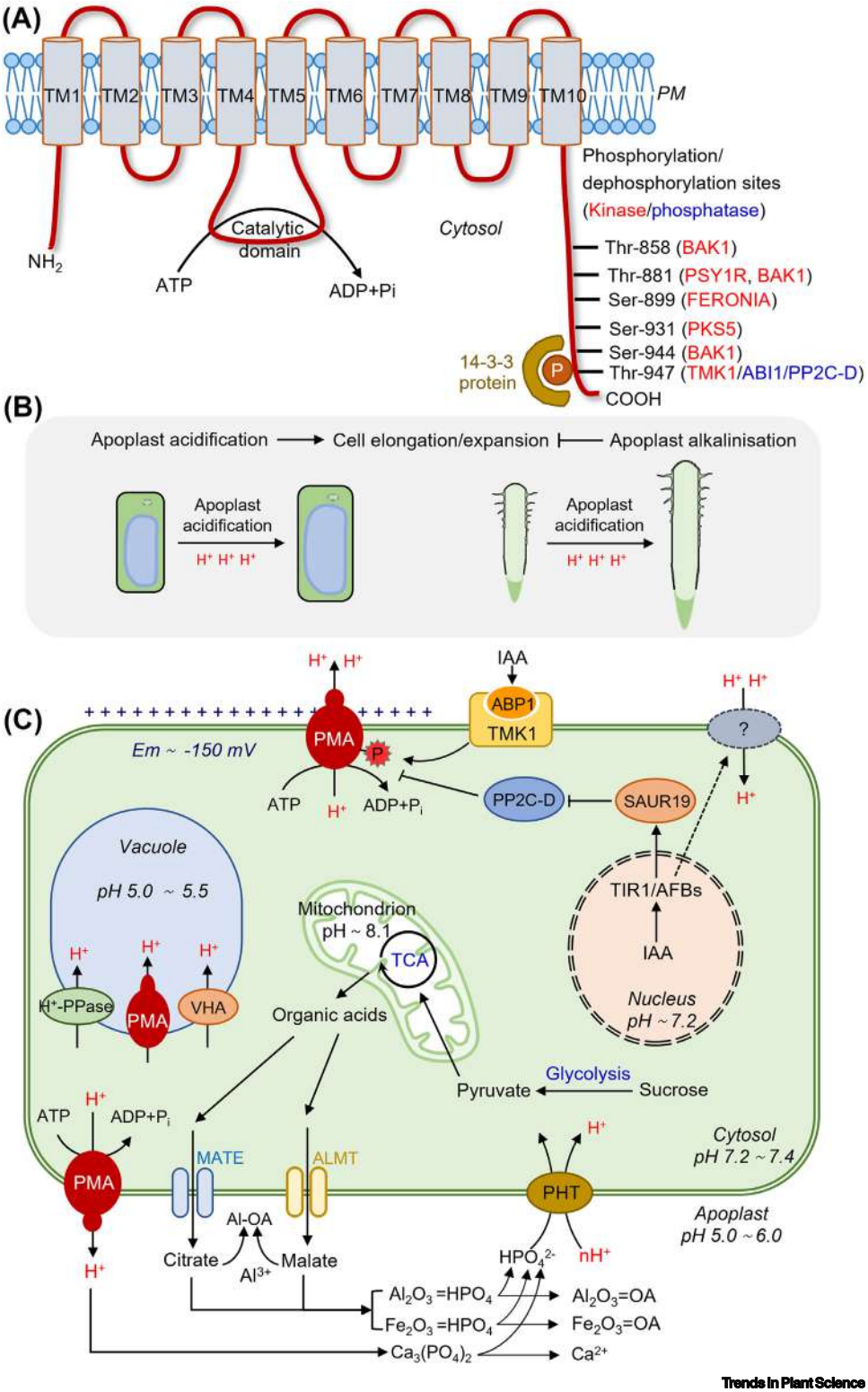

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the catalytic hydrolysis of the enzyme, while the hydrophilic N terminal and C terminal mainly regulate the activity of the enzyme [15]. PMA exists extensively in plants and fungi, but not in animals, although animals possess Na^+/K^+ pumps that are structurally similar to PMA [16].

As a key enzyme in plants, PMA plays indispensable roles in various physiological activities by generating membrane potential, as well as providing proton motive force for the uptake of nutrients and the transport of metabolites [1,17,18]. The active transport of various nutrients and metabolites mediated by different types of transporters localized on the PM usually depends on an H^+ electrochemical gradient across the PM, which is generated by PMA (also known as proton motive force). It has been well documented that PMA plays important roles in regulating cell growth, pollen tube elongation, stomatal opening, nutrient uptake, sugar transport, and environmental stress responses (such as nutrient deficiency, salinity, drought, and pathogen infection) [1,18–23]. In addition, the classical **acid-growth theory** assumes that auxin promotes the pumping of H^+ from the cytoplasm by stimulating PMA activity, and leads to **apoplast acidification** causing cell wall relaxation, which facilitates cell elongation and expansion [24,25] (Figure 1B).

Post-translational and transcriptional regulation of PMA

There are two different states in the terminal domains of PMA: the autoinhibited basal state, with a relatively loose connection between ATP hydrolysis and H^+ transport, and the activated state in which ATP hydrolysis is tightly coupled to H^+ transport. Regulation of PMA activity is implemented mainly by a change in the spatial structure of the N terminal and C terminal autoinhibitory domain, thus activating or inhibiting its proton pump activity [15]. Post-translational modifications such as **protein phosphorylation** and **protein dephosphorylation** play crucial roles in the regulation of PMA activity [15]. Phosphorylation of Ser-899 and Ser-931 in arabidopsis AHA2 reduces proton pump activity, while phosphorylation of Thr-881 and Thr-947 increases proton pump activity [26–29] (Figure 1A). Phosphorylation of the penultimate Thr of PMA can promote the binding of 14-3-3 proteins, which further relieves the self-inhibition of the C terminal of PMA to activate the proton pump activity [30–33]. Phosphorylation of some amino acid residues in PMA can activate the proton pump activity independently of 14-3-3 proteins, such as the phosphorylation of tobacco PMA2 at Thr-889 [34]. However, phosphorylation of some amino acid residues, such

Figure 1. Schematic structure of a plasma membrane H^+-ATPase (PMA) protein and the role of PMAs in cellular growth and organic acid exudation in roots. (A) Schematic presentation of the structure of arabidopsis AHA2. This protein is integrated into plasma membrane (PM) by ten transmembrane domains (TM1–TM10) and has most of its remaining mass – including the catalytic domain, the N and C terminal domains – exposed on the cytosolic side of the membrane. The activity of AHA2 is regulated at post-translational level by protein phosphorylation/dephosphorylation at several sites in the C terminal auto-inhibitory domain. Thr-881, Ser-889, and Ser-931 can be phosphorylated by PSY1R, FERONIA, and PKS5 kinases, respectively. Phosphorylation of the penultimate Thr-947, which is mediated by TMK1, can activate PMA activity by promoting the binding of 14-3-3 proteins, while PP2C-Ds and ABI1 are likely to mediate dephosphorylation of AHA2 at Thr-947. (B) Schematic presentation of acid growth. Cell elongation is promoted by apoplast acidification and inhibited by apoplast alkalinization. (C) Schematic presentation of the involvement of PMAs in auxin-regulated root growth and in phosphate acquisition facilitated by organic acid exudation. In acid growth, the TIR1/AFB-mediated auxin signaling induces the expression of SAUR19 that inhibits PP2C-D protein phosphatases, thus facilitating H^+ efflux through PMAs and cell-wall acidification. In roots, the intracellular TIR1/AFB-mediated pathway promotes H^+ influx by uncharacterized transporters, leading to apoplast alkalinization and root growth inhibition. Extracellular auxin signal induces phosphorylation of PMA at the penultimate Thr mediated by TMK1 that is in complex with auxin-binding protein ABP10, and thus activates PMA activity. PMA is involved in the exudation of organic anions, which solubilize inorganic and organic P pools. Abbreviations: ADP, adenosine diphosphate; AFBs, auxin signaling F-box proteins; ALMTs, aluminum-activated malate transporters; ATP, adenosine triphosphate; BAK1, brassinosteroid-insensitive 1-associated receptor kinase 1; EM, resting membrane potential; FERONIA, the receptor-like kinase1-like family member protein; H^+-PPase, H^+-pyrophosphatase; IAA, indoleacetic acid; MATEs, multidrug and toxic compound extrusions; PHTs, phosphate transporters; Pi, inorganic phosphate; PKS5, SOS2-like protein kinase, also named CBL-interacting protein kinase 11 (CIPK11); PSY1R, PSY1 receptor protein kinase; Ser, serine; TCA, tricarboxylic acid cycle; TIR1, transport inhibitor response protein 1; Thr, threonine; TM, transmembrane domain; TMK1, transmembrane kinase 1; TMKs, transmembrane kinases; VHA, vacuolar H^+-ATPase.

Glossary

Acid-growth theory: a theory explaining the expansion dynamics of cells and organs in plants by involving auxin-induced apoplastic acidification.
Apoplast acidification: H^+ extrusion into the apoplast, the space from the external face of plasma membrane to the cell wall (including the cell wall), leading to the decrease of apoplast pH which is essential for cell elongation and expansion.
Arbuscular mycorrhiza (AM): a widespread symbiosis between mycorrhizal fungi and the root systems of most land plants; highly branched haustoria-like structures are formed in the cortical root cells and provide essential mineral nutrients for host plants in exchange for organic carbon.
Biological nitrification inhibitor: substances released from plant roots that can repress nitrifier activity in soils, reduce nitrogen loss associated with nitrification and denitrification, and thus enhance nitrogen use efficiency.
Depolarization: a change inside a living cell that causes the distribution of electric charges to alter, leaving the cell with a less negative charge than the outside.
Membrane potential: the electrical potential difference between the interior and the exterior of a living cell.
Periarbuscular membrane: a plant membrane that is generated by the root cell to surround the arbuscule for nutrient exchange during arbuscular mycorrhizal symbiosis. Symbiosis-specific transporter proteins are localized in the periarbuscular membrane.
Plasma membrane (PM) H^+-ATPase (PMA): a transmembrane enzyme that hydrolyzes ATP to pump H^+ outside the plant cell, which in turn generates a transmembrane H^+ gradient.
Protein dephosphorylation: the removal of a phosphate group from an amino acid residue of a protein through hydrolysis mediated by a protein phosphatase.
Protein phosphorylation: a reversible post-translational modification of proteins in which an amino acid residue is phosphorylated by a protein kinase, resulting in the addition of a covalently bound phosphate group.
Proton motive force: the force that results from an electrochemical gradient of protons across a membrane that can be used for the various energy-consuming transmembrane transportations required by a living cell.

as the phosphorylation of tobacco PMA2 at Thr-931 and Ser-938, hinders the binding of 14-3-3 protein to PMA, and thus negatively regulates the proton pump activity [29,35]. In addition, some amino acid residues of PMA interact with 14-3-3 proteins in a phosphorylation-independent manner, such as Thr-924 in AHA2 [36]. Notably, Thr-881 phosphorylation in response to red light and blue light in guard cells has been recently reported to play a significant role for light-induced stomatal opening [166,167]. Phosphorylation of PMA is mediated by protein kinases, while dephosphorylation of PMA is mediated by protein phosphatases. The combined action of these two kinds of enzymes determines the PMA proton pump activity [1,37,168]. At present, a variety of protein kinases and phosphatases have been identified to regulate PMA mainly at amino acid residues in the C terminal autoinhibitory domain [38,39]. Protein kinases (such as PKS5, FERONIA, PSY1R, TMK1 and BAK1 [26,27,29,40–42]) and phosphatases (such as PP2C-D and ABI1 [43–46]) are involved in the regulation of phosphorylation or dephosphorylation of PMAs (Figure 1A). Various signals – including plant hormones such as auxin and abscisic acid [22,28,39], mycotoxins such as fusicoccin and tenuazonic acid [47], signaling peptides such as peptide containing sulfated tyrosine 1 (PSY1) and rapid alkalization factors (RALFs) [26,27], lipids [48], and environmental factors such as light, salt-alkali, nutrients, and pathogens [33,49–51] – can regulate PMA activity by mediating the phosphorylation or dephosphorylation of PMAs.

Proton pump: includes H^+-ATPase and H^+-PPase, which both extrude H^+ against the transmembrane H^+ gradient, thereby establishing a membrane potential and providing proton motive force.

PMA activity is also regulated at the transcriptional level. The expression of several PMA genes is affected by environmental factors such as low pH, salt, hypoxia, heavy metals, and nutrients [52–57]. Only a few upstream transcription factors of PMA genes have been identified. For example, WRKY1 is involved in salt tolerance by positively regulating HA1 in *Populus euphratica* [58], and MYB308 is involved in iron (Fe) deficiency tolerance by positively regulating Fe deficiency-inducible HA6 in citrus [59]. For the spatial expression patterns, some PMA genes are extensively expressed in diverse plant tissues, and some are expressed in specific tissues. For example, rice *OSA7* is constitutively expressed in leaves, roots, guard cells, and mesophyll cells [60], while rice *OSA9* is specifically expressed in roots [60], and arabidopsis *AHA3* is dominantly expressed in the phloem of vascular tissue [61]. PMA genes that are specifically expressed in certain tissues may execute tissue-specific functions. However, the molecular mechanisms underlying the tissue-specific and stress-responsive expression patterns of PMA genes still need to be explored.

Role of PMA in root growth for nutrient acquisition

Plant roots, especially lateral roots and root hairs, are indispensable organs for nutrient acquisition. PMA is involved in the regulation of root system architecture in response to nutrient signals. The expression of *AHA2* is induced by low nitrogen (N) in arabidopsis, and the length of primary roots and lateral roots is decreased in the knockout mutant of *AHA2* under different N levels [62]. Arabidopsis *AHA2* and *AHA7* are predominantly expressed in root epidermal cells; AHA2 positively regulates primary root growth, possibly by promoting cell expansion, but AHA2 and AHA7 negatively regulate root hair elongation [63]. Short and highly branched lateral roots are formed under a local supply of ammonium (NH_4^+) in arabidopsis [64]. Recently, it has been revealed that NH_4^+-uptake-induced and PMA-mediated acidification of the root apoplast increases pH-dependent diffusion of protonated auxin into cortical and epidermal cells overlaying lateral root primordia, and subsequently facilitates the emergence of lateral roots [65]. Under local supply of NH_4^+, knockout mutation of *AHA2* reduces the third-order lateral root density and the second- and third-order lateral root length [65].

The induction of root hair formation by exogenous methyl jasmonate is related to the increased PMA activity and rhizosphere acidification in seedlings of lettuce (*Lactuca sativa*) [66]. In general, auxin promotes shoot growth (such as hypocotyl elongation) but inhibits root growth. Recent studies have demonstrated that intracellular auxin inhibits root growth by promoting H^+ influx

and apoplast alkalinization through the TIR1/AFB signaling pathway, while extracellular auxin signaling induces phosphorylation of AHA2 at Thr-947 through TMK1 and activates PMA activity [40,41] (Figure 1).

Role of PMA in N acquisition and utilization

N is the most abundant and most important mineral nutrient in plants. Plants mainly absorb inorganic N from soil in forms of ammonium (NH_4^+) and nitrate (NO_3^-). NO_3^- is co-transported with H^+ into cells by a symporter, and the stoichiometry of NO_3^- and H^+ is about two [67,68]. Nitrate transporter NRT1 and NRT2 are NO_3^-/H^+ co-transporters that transport one NO_3^- with two H^+ [69] (Figure 2). The absorption of NO_3^- usually leads to **depolarization** of the membrane potential, which is compensated by the activation of PMA to repolarize and maintain the membrane potential [70–72]. It has been documented that NO_3^- uptake by NRTs in roots is associated with an H^+ gradient across the PM in plants [73,74]. NO_3^- could induce the activity of PMA [75], and consistently, inhibitors of PMA could reduce the uptake of NO_3^- in maize roots [76]. By contrast, coumarin, one of the simplest plant secondary metabolites, could promote the high-affinity uptake of NO_3^- by increasing PMA activity in the roots of maize (*Zea mays*) [73]. The expression of PMA genes, such as *MHA3* and *MHA4* in maize roots [77], and *VvHA2* and *VvHA4* in grape (*Vitis vinifera*) roots [78],

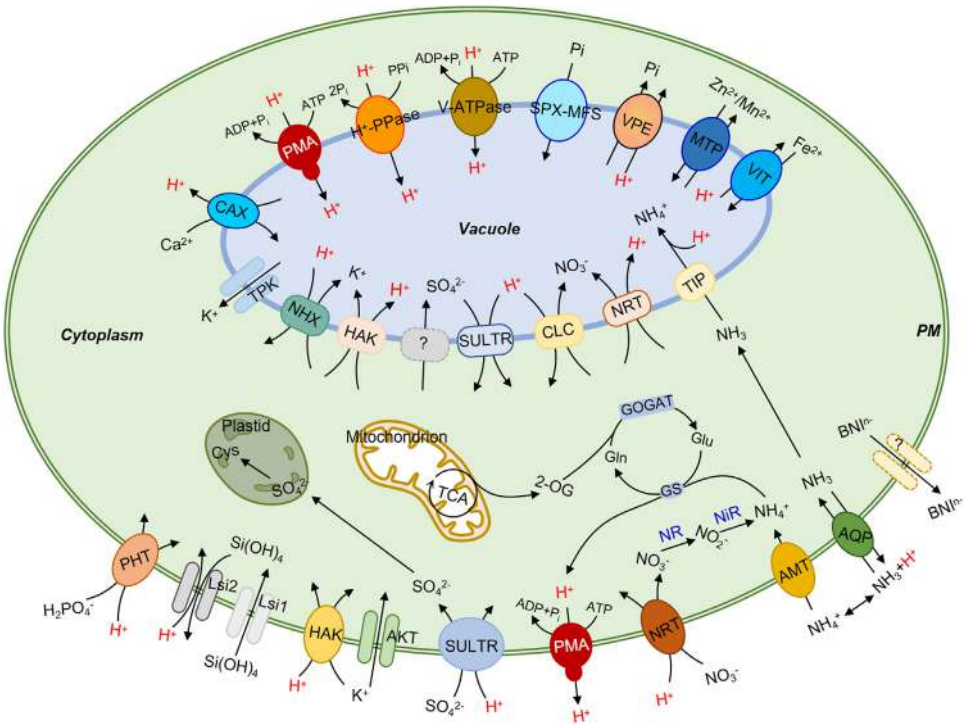

Figure 2. Roles of plasma membrane H^+-ATPases (PMAs) in the uptake and/or assimilation of mineral nutrients by providing proton motive force or membrane potential for various nutrient transporters. Abbreviations: AKT, arabidopsis K^+ transporter; AMT, ammonium transporter; AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; AQP, aquaporin; BNI, biological nitrification inhibitor; CLC, chloride channel; Gln, glutamine; Glu, glutamate; GOGAT, glutamate synthase; GS, glutamine synthetase; HAK, high-affinity potassium transporter; Lsi, Low silicon; MTP, metal tolerance protein; NHX, Na^+/H^+ antiporter; NiR, nitrite reductase; NR, nitrate reductase; NRT, nitrate transporter; 2-OG, 2-oxoglutarate; SPX-MFS, SYG1, PHO81, and XPR1 (SPX), major facility superfamily (MFS) protein; SULTR, sulfate transporter; TCA, tricarboxylic acid cycle; TIP, tonoplast integral protein; TPK, two-pore K^+ channel; VIT, vacuolar iron transporter; VPE, vacuolar phosphate efflux transporter.

is induced by NO_3^- supply in root media. The expression of *MHA1* in low-N-tolerant varieties is significantly higher than that in low-N-sensitive varieties in maize [79], suggesting a potential role of MHA1 in N use efficiency. In addition, resupply of NO_3^- to rice grown under N deficiency significantly induces the expression of *OsA2*, *OsA5*, *OsA7*, and *OsA8* [80]. Knockdown of *OSA2* expression by artificial microRNA represses the uptake of NO_3^- in rice roots under low NO_3^- conditions, and results in significant decreases in leaf N concentration and grain yield [81]. Notably, NO_3^- transport in plant leaves is also associated with PMA activity; in cucumber leaves, the expression of a PMA gene, *CsHA2*, is induced by NO_3^- incubation [82]. Recently, it has been indicated that light-induced NO_3^- uptake by leaves is associated with the activation of PMA by photosynthesis and its resulting sugar metabolites in plant leaves [23]. Therefore, the uptake of NO_3^- is closely related to PMA in both roots and leaves, and PMA is potentially involved in plant adaptation to low N stress by promoting NO_3^- absorption from root media or soils, but further studies are required to explore the underlying molecular mechanisms.

The uptake of NH_4^+ by roots is also related to PMA. Plant roots absorb NH_4^+ predominantly through ammonium transporters (AMTs). Vanadate, an inhibitor of PMA, could eliminate the depolarization of membrane potential caused by NH_4^+ uptake in rice roots [83] and significantly inhibit NH_4^+ uptake, while fusicoccin, a PMA activator, could significantly increase NH_4^+ uptake rate in rice roots [84]. Knockout of *OSA1* significantly decreases NH_4^+ uptake by rice roots, while overexpression of *OSA1* significantly increases H^+ efflux and NH_4^+ uptake [84]. Most of the NH_4^+ taken up by roots is quickly assimilated in root plastids or shoot chloroplasts by the glutamine synthetase/glutamate synthase (GS/GOGAT) cycle, and two H^+ are produced for the assimilation of one NH_4^+ [69]. Generally, the supply of NH_4^+-N nutrition leads to strong rhizosphere acidification, while NO_3^--N nutrition leads to rhizosphere alkalinization [85–87]. It has been revealed that the ability of rice plants to prefer NH_4^+ nutrition is related to the great capability of roots to promote PMA activity to adapt to the rhizosphere acidification associated with NH_4^+ nutrition [53].

PMA also plays an important role in regulating N assimilation. After uptake by plant roots, NO_3^- is first reduced to NO_2^- by nitrate reductase (NR), then to NH_4^+ by nitrite reductase (NiR), and subsequently NH_4^+ is assimilated into amino acids through the GS/GOGAT cycle in the cytosol and plastids of roots or in the cytosol and chloroplasts of shoots. Reduction of one NO_3^- to one NH_4^+ consumes two H^+ in the cytosol [69]. Therefore, NO_3^- assimilation as an H^+-consuming process leads to an increase in cytoplasmic pH, which compensates for the H^+ influx accompanied by NO_3^- uptake. The assimilation of one NH_4^+ produces two H^+ in the cytosol; thus, if the excess H^+ is not pumped out in time, the cytoplasm will be acidified rapidly and cause NH_4^+ toxicity [88]. Overexpression of *OSA1* increases the expression of GS genes (*GS1;2* and *GS2*) and GOGAT genes (*NADH-GOGAT1*, *NADH-GOGAT2* and *Fd-GOGAT*) in rice roots and leaves [84], suggesting that overexpression of *OSA1* could enhance the activities of GS and GOGAT, which may further promote the conversion of NH_4^+ into amino acids in roots [89]. Therefore, PMA is involved in the coordination of NH_4^+/NO_3^- uptake and NH_4^+/NO_3^- assimilation, which are two closely related biological processes in N metabolism.

Soil nitrification results in N loss from agricultural soil and subsequent environmental pollution [90]. The roots of certain plants can inhibit the activity of nitrifying bacteria by secreting organic compounds such as brachialactone, methyl 3-(4-hydroxyphenyl) propionate, and 1, 9-decanediol. These substances are named **biological nitrification inhibitors (BNIs)**, and they are closely associated with N use efficiency in plants [91–94]. There are two types of BNIs: hydrophilic and hydrophobic. It has been shown that NH_4^+-N nutrition promotes the secretion of hydrophilic BNIs in a PMA-dependent manner as compared with NO_3^--N nutrition [55,91]. Vanadate inhibits

the secretion of BNIs from sorghum roots, while fusicoccin promotes the secretion of BNIs [95]. The secretion of brachialactone, a hydrophilic BNI, depends on the proton motive force provided by PMA in *Brachiaria humidicola* roots [96]. In addition, the NH_4^+-induced release of 1,9-decanediol by rice roots is also related to NH_4^+-mediated rhizosphere acidification [97], suggesting that PMA is also involved in the release process of 1,9-decanediol. Therefore, it is hypothesized that hydrophilic BNIs are secreted from the cytoplasm through unknown anion channels, which are dependent on the membrane potential established by PMAs (Figure 2).

Role of PMA in organic anion exudation and phosphate acquisition

As an indispensable component of nucleic acids and phospholipids, phosphorus (P) plays essential roles in plant growth and metabolism. Monohydrogen phosphate (HPO_4^{2-}) and dihydrogen phosphate ($H_2PO_4^-$) are usually the available inorganic phosphate (Pi) forms taken up by plant roots through phosphate transporters (PHTs). PHT is a cotransporter associated with H^+ influx, usually transporting one Pi molecule accompanied by two to four H^+ [98]. Therefore, the uptake of Pi, especially at low concentrations (through high-affinity PHTs), is dependent on PMA to provide proton motive force. Under conditions of low P, the activity of PMA is significantly increased in the roots of plants such as white lupin (*Lupinus albus*), soybean, and rice [99–101]. Exogenous application of chemicals that regulate PMA activity significantly influences Pi uptake [102,103]. NH_4^+-N can increase Pi uptake by rice roots by promoting PMA activity [102]. It has been found that overexpression of *qPE9-1* encoding a γ subunit of rice GTP-binding proteins (G proteins) increases the concentration of soil-available Pi in rice rhizosphere by enhancing PMA activity and promoting root sheath formation [104]. In arabidopsis roots, the expression of *AHA2* and *AHA7* is induced by low P, and these two PMAs participate in the regulation of the growth of both primary roots and root hairs by increasing H^+ efflux [105]. Therefore, PMA is involved in plant adaptation to low P conditions by providing proton motive force and regulating root growth to promote Pi acquisition from soils. The acidification of the rhizosphere and organic anion exudation promoted by PMAs can also facilitate Pi acquisition by improving Pi availability in soils. However, the molecular mechanism underlying low P-induced activation of PMA is still unclear. Whether and how PMA is regulated at transcriptional and post-translational levels deserve further investigation.

Usually Pi is adsorbed by Fe and Al oxides in acidic soils, and is precipitated by Ca in neutral or alkaline soils. Thus the plant-available Pi in soils is very low. Low P induces the exudation of organic anions such as malate, citrate, and oxalate from plant roots [106–109]. The exudation of organic anions is an important strategy to promote Pi acquisition by plant roots, because organic anions can mobilize the inorganic P pools by forming complexes with Al and Fe oxides/hydroxides [110,111]. The secretion of organic anions from roots is mediated by organic anion transporters or channel proteins located on the PM, such as aluminum (Al)-activated malate transporter (ALMT) and multidrug and toxic compound extrusion (MATE) transporter [110] or anion channels (Figure 1C). Studies have shown that citrate secreted from roots is transported across the PM by MATE transporters that are coupled with H^+ influx [112,113]. The induction of citrate exudation by low P is also associated with PMA, because the activity of PMA and citrate exudation in the proteoid roots of white lupin are both enhanced under low P conditions, and the H^+ and citrate exudation are increased by PMA activators but are repressed by PMA inhibitors [100,108,114]. Therefore, citrate exudation induced by P deficiency is dependent on the activity of PMA that provides a membrane potential, releases H^+ to dissolve Pi that is dissipated by Ca in soils, and also maintains cytoplasmic pH which may be acidified by organic acid biosynthesis. The exudation of malate is also associated with PMA-mediated H^+ efflux, based on the evidence that malate secretion is positively correlated with H^+ secretion in proteoid roots of white lupin under P deficiency, and that both malate and H^+ efflux are promoted by fusicoccin but are inhibited by vanadate [108].

Although Al is not an essential mineral nutrient, Al toxicity has been known to be a major limiting factor impairing plant growth in acidic soils [106]. Organic anions secreted by roots can chelate Al in the rhizosphere, reducing its toxicity (Figure 1C). Knockout mutation of *AHA2* results in reduced Al resistance, which is attributed to lower malate exudation in arabidopsis [115]. Recently, it has been found that PP2C-D phosphatases negatively regulate PMA activity by mediating protein dephosphorylation, and play a contrasting role in Al resistance in rice and arabidopsis. In arabidopsis, mutations of genes encoding PP2C-D5/D6/D7 phosphatases increase Al resistance because of enhanced malate secretion, while in rice, the PP2C-D phosphatase SAL1 positively regulates Al resistance by inhibiting PMA activity via dephosphorylation and restricting PMA-promoted Al uptake [115]. However, some studies showed that the secretion of organic anions is independent of PMA. In tomato roots, Al-induced oxalate secretion is independent of PMA activity [114,116]. Al-induced citrate secretion in proteoid roots of white lupin is also independent of PMA activity, but is associated with K^+ efflux [117]. These results suggest that the role of PMA in Al-induced organic anions exudation and Al resistance is different in different plant species.

Role of PMA in potassium (K) acquisition

K is an essential macronutrient playing important roles in maintaining enzyme activity and regulating osmotic potential. K^+ is the most abundant cation that has no negative effect on plant growth compared with other cations. The transmembrane transport of K^+ is strongly dependent on membrane potential, which is generated by PMA. It has been documented for many years that K^+ uptake is closely related to H^+ efflux [118]. KUP/HAK/KT family transporters – found in prokaryotes, fungi, and plants – mediate K^+ uptake through a K^+/H^+ symport mechanism [119] (Figure 2). Application of exogenous chemical modulators (such as methylpyraquine chloride, an inhibitor of gibberellin biosynthesis), enhances K^+ uptake by promoting PMA activity in cotton (*Gossypium hirsutum*) roots [120]. In addition, it has been found that salt stress can stimulate PMA activity to promote K^+ uptake or inhibit K^+ efflux, thus increasing K content in plants to adapt to salinity [121,122]. Interestingly, halophyte species and salt-tolerant cultivars typically retain more negative membrane potential values due to intrinsically higher PMA activity [123,124]. Increased PMA-mediated H^+ extrusion could energize Na^+ efflux though the Na^+/H^+ exchanger and reduce the magnitude of PM depolarization to prevent K^+ loss through depolarization-activated K^+ outward-rectifying channels, thus improving plant adaptation to salinity in the expense of energy consumption.

PMA is also involved in plant responses to low K stress. PMA activity is induced by K^+ deficiency, which could enhance H^+ efflux and apoplast acidification [118], and thus provide higher proton motive force for K^+ uptake in plants [125]. External NH_4^+ addition can increase K^+ uptake by promoting PMA activity and the resulting H^+ efflux under low K conditions [126]. K^+ can bind to the site containing Asp-617 in the cytoplasmic phosphorylation domain of PMA and negatively regulate the transmembrane electrochemical potential by uncoupling ATP hydrolysis and H^+ efflux [127], but whether the negative regulation is repressed under low K conditions is still unclear. Recently, it has been found that receptor-like protein kinase brassinosteroid-insensitive 1-associated receptor kinase 1 (BAK1) is involved in the low K response by positively regulating the activity of AHA2 in arabidopsis. BAK1 interacts with the C terminus of AHA2 and phosphorylates Thr-858 and Thr-881, leading to the enhancement of proton pump activity of AHA2 and the subsequent K^+ uptake under low K condition [128]. Interestingly, K^+ addition could also activate PMA under certain conditions. Under high NH_4^+ conditions, increasing K^+ concentration in the nutrient solution can inhibit NH_4^+ absorption and reduce NH_4^+ toxicity by promoting the activity of PMA in rice roots [129,130]. Overexpression of a high-affinity K^+ transporter *OsHAK5* increases PMA activity and polar auxin transport in rice roots [131]. However, the molecular

mechanism underlying the linkage between PMA and K^+ transporters induced by low K, such as KUP/HAK/KT and HKT, is still ambiguous. Further studies are required to explore the complicated interplays between K^+ nutrition and PMA regulation.

The close linkage between PMA and K^+ transport plays an important role in guard cells for stomatal movement. Activated PMA increases H^+ efflux and then causes membrane hyperpolarization, which further activates K^+ channel KAT and leads to K^+ influx and stomatal opening by facilitating water uptake and swelling of guard cells [132,133]. On the contrary, when PMA activity is inhibited, the resulting depolarization of membrane potential leads to activation of guard cell outwardly rectifying K^+ channel (GORK) to boost stomatal closure [134,135].

Role of PMA in iron (Fe) acquisition

As one of the essential micronutrients in plants, Fe acts as a cofactor for enzymes and plays critical roles in chlorophyll biosynthesis and photosynthesis. Although the content of Fe in soil is high, most of the Fe is in the form of Fe oxide or hydroxide that cannot be directly absorbed and utilized by plants. Fe deficiency is a major limiting factor in soil affecting plant growth and development, especially in alkaline or calcareous soils. The availability of Fe in soil is highly correlated with pH; it is increased with a decrease in soil pH [136]. According to the mechanism of Fe uptake in plants, plants can be divided into two kinds: strategy I and strategy II [87]. In strategy I plants (all spermatophytes with the exception of gramineous species), the central component of Fe acquisition is the reduction of Fe(III) chelates by an inducible PM-localized oxidoreductase, and the following uptake of released ferrous ion. In most strategy I plants, Fe reduction is accompanied by PMA-mediated H^+ extrusion, which weakens the Fe–O bond of Fe(III) oxides and leads to metal detachment [137] (Figure 3). Strategy II plants (gramineous species) rely on the secretion of phytosiderophores of the mugineic acid family that are increased upon Fe deficiency and form complexes with Fe(III) [138,139]. The loaded Fe(III)–phytosiderophore complex is taken up by root cells directly, without reduction into ferric Fe. Strategy I is characterized by high-valent Fe reduction and divalent Fe absorption, and is commonly found in dicotyledonous and non-gramineous monocotyledonous plants. Strategy I plants increase PMA activity under Fe deficiency in order to increase Fe availability by secreting H^+ to acidify the rhizosphere. So far, there is no record of Fe deficiency-induced H^+ secretion in strategy II plants, suggesting that the rhizosphere Fe activation mechanism based on H^+ secretion is unique to strategy I plants [140].

Under conditions of Fe deficiency, the expression of *AHA2*, *AHA3*, *AHA4*, and *AHA7* is induced, and the amount of AHA proteins is significantly increased in arabidopsis, where AHA2 plays a predominant role in the rhizosphere acidification to enhance Fe acquisition [52]. Chrysanthemum basic helix–loop–helix 1 (CmbHLH1), a transcription factor from chrysanthemum, positively regulates Fe uptake under Fe deficiency by enhancing the expression of *CmHA* and the subsequent rhizosphere acidification [141]. Two transcription factors, *MbERF4* and *MbERF72*, which are induced by Fe deficiency, interact with each other and synergistically inhibit the expression of *MbHA2* in apple (*Malus baccata*) [142]. The expression of *MxERF4* and *MxERF72* is reduced in Fe-deficiency-tolerant apple species (*Malus xiaojinensis*) when compared with Fe-deficiency-sensitive apple species (*M. baccata*) under Fe deficiency, leading to the increased expression of PMA gene *MxHA2* and higher tolerance to Fe deficiency [142]. *MxHA2* is positively regulated by a bHLH transcription factor MxbHLH104, which is phosphorylated and enhanced by a MAP kinase (MxMPK6-2) at Ser169 [143]. MxMPK6-2 interacts with and phosphorylates MxHA2 at Ser-909, site of its C terminus, and at Thr-320 and Thr-412, sites of its catalytic domain; phosphorylation at Ser-909 and Thr-320 could promote the activity, while phosphorylation at Thr-412 may inhibit the activity of MxHA2 [143] (Figure 3). In addition, a calmodulin-binding

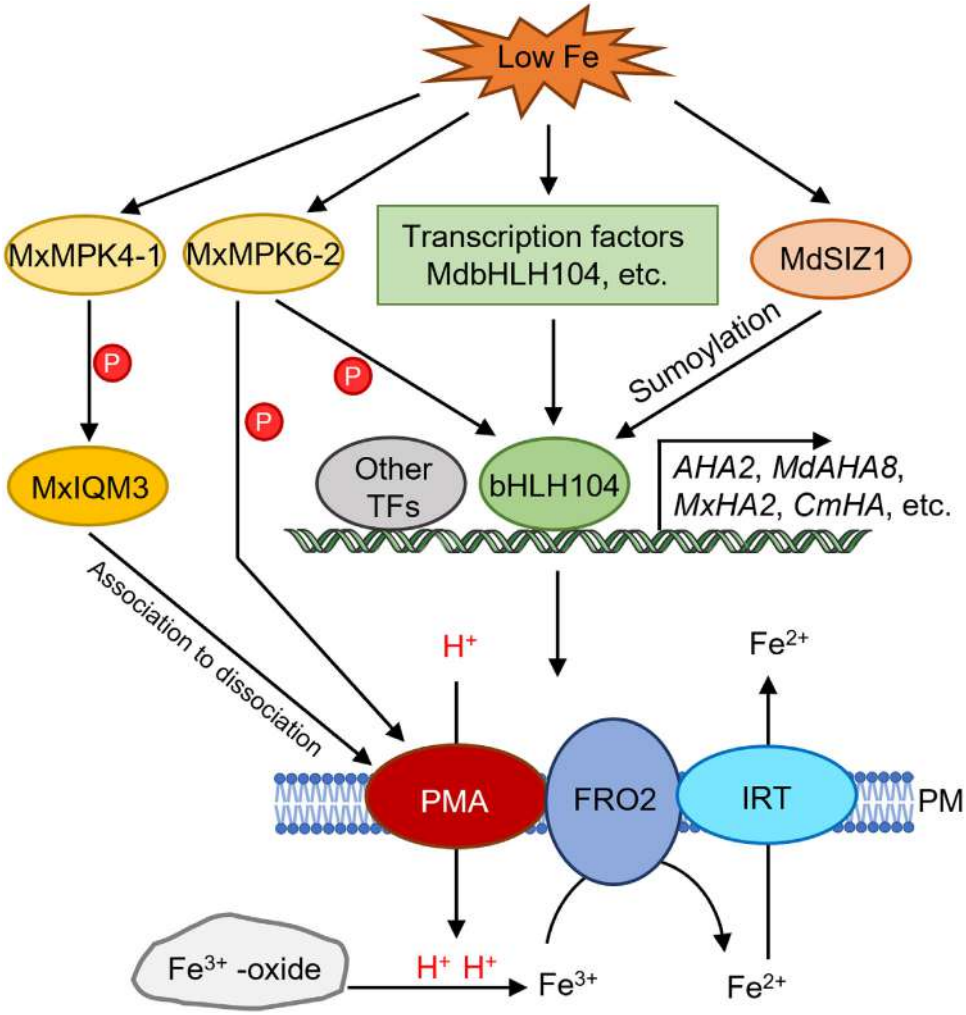

Figure 3. Roles of plasma membrane H^+-ATPases (PMAs) in iron (Fe) absorption in strategy I plants. Under low Fe conditions, transcription factors (such as MdbHLH104), are activated and the expression of downstream PMA genes, such as *MdAHA8* and *MxHA2*, are induced. In apple, the sumoylation of bHLH104, which is mediated by SIZ1, promotes the protein stability, and the phosphorylation of bHLH104 at Ser-169 by MxMPK6-2 enhances the protein stability. Apple MxMPK6-2 also regulates PMA activity by mediating phosphorylation of MxHA2 under Fe deficiency. Apple MxIQM3 interacts with MxHA2 under Fe sufficiency. Under Fe deficiency, MxIQM3 is phosphorylated by MxMPK4-1 at Ser-393, and the phosphorylated MxIQM3 dissociates from MxHA2 to promote H^+ secretion. Thus, under low Fe stress, PMA-mediated H^+ secretion is enhanced, and the resulting rhizosphere acidification increases the solubility of ferric hydroxide ($Fe(OH)_3$). Ferric Fe is then reduced by ferric reduction oxidase 2 (FRO2) reductase to Fe^{2+} and transported into cells by the zinc-regulated, iron-regulated transporter like protein (ZIP) transporter IRON-REGULATED TRANSPORTER 1 (IRT1). Abbreviations: PM, plasma membrane; TF, transcription factor.

IQ-motif-containing protein 3 (IQM3) interacts with MxHA2 and represses MxHA2-mediated H^+ efflux in *M. xiaojinensis*. Interestingly, IQM3 is phosphorylated by MxMPK4-1 at Ser-393 under conditions of Fe deficiency, and this phosphorylation impairs the interaction between IQM3 and MxHA2 and thus promotes H^+ secretion and Fe deficiency tolerance [144]. MdbHLH104 from other apple species (*Malus domestica*) also positively regulates Fe deficiency tolerance by promoting the expression of *MdAHA8* and H^+ secretion; overexpression of *MdbHLH104* increases PMA activity and Fe concentration in apple seedlings under Fe deficiency [145]. Interestingly,

MdbHLH104-mediated regulation of PMA activity in response to Fe deficiency is positively regulated by MdSIZ1, which is a small ubiquitin-like modifier (SUMO) E3 ligase that is induced by Fe deficiency and inhibits the ubiquitin-mediated protein degradation of MdbHLH104 to increase the activity of PMA and thus promotes Fe uptake [146] (Figure 3).

Role of PMA in arbuscular mycorrhizal symbioses

To facilitate nutrient acquisition, most terrestrial plants are able to form a mutualistic symbiosis with arbuscular mycorrhizal fungi (AMF) (**arbuscular mycorrhiza, AM**). AMF helps plants to acquire nutrients through the greatly extended hypha system, and in exchange plants provide carbohydrates for the AMF. The deficiency of mineral nutrients in soils, especially P, promotes the beneficial symbiosis with AMF in plant roots in order to obtain more nutrients [147]. The uptake of nutrients by a mycorrhizal plant occurs at a specialized interface formed in arbuscule-colonized cortical cells. Arbuscules are always enveloped in a plant-derived PM (i.e., the **periarbuscular membrane**) which separates the fungal hyphae from the host cytoplasm (Figure 4). A group of P, N, and K transporters that are induced by AMF and localized in arbuscule-colonized cortical roots have been demonstrated to be involved in nutrient uptake of the mycorrhizal pathway in plant roots. For example, several Pi transporters belonging to the PHT1 family (Pi/H^+ symporter) have been identified to

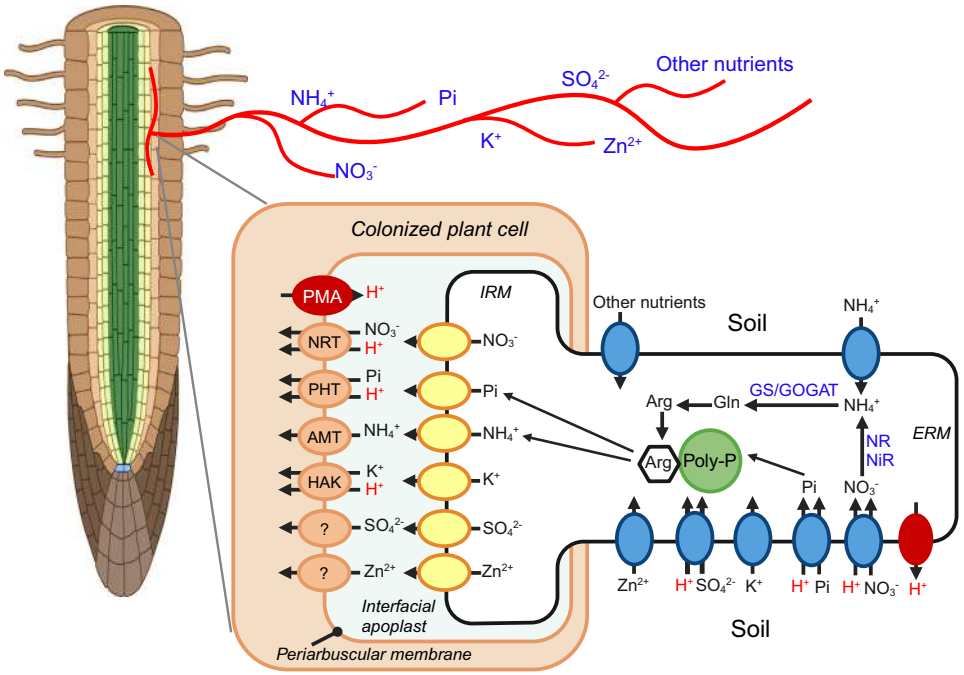

Figure 4. Roles of plasma membrane H^+-ATPases (PMAs) in the uptake of nutrients through arbuscular mycorrhizal (AM) symbioses. In AM symbiosis, nutrient ions such as NH_4^+, NO_3^-, Pi, K^+, Zn^{2+}, and SO_4^{2-} are absorbed by the extraradical mycelium (ERM) from soil solution through various fungal transporters. Inorganic phosphate (Pi) absorbed by the ERM is transported mainly in the form of polyphosphate (Poly-P) granules. The NO_3^- absorbed by the ERM can be reduced and converted into NH_4^+ by sequential action of nitrate reductase (NR) and nitrite reductase (NiR). NH_4^+ absorbed by the ERM can be assimilated into glutamine (Gln), then into arginine (Arg) via the glutamine synthetase–glutamate synthase (GS-GOGAT) pathway and translocated, probably coupled with Poly-P, through the intraradical hyphae. Nutrients absorbed by the ERM are also directly translocated into the intraradical mycelium (IRM) and released into the interfacial apoplast, and are subsequently imported into colonized root cells by corresponding transporters that are located on the periarbuscular membrane (PAM). PMAs located at the PAM provide proton motive force for the uptake of nutrients, such as Pi, NO_3^-, and K^+. Abbreviations: AMT, ammonium transporter; HAK, high-affinity potassium transporter; NRT, nitrate transporter; PHT, phosphate transporter. Figure created with BioRender.

be essential for symbiotic Pi uptake, such as *Medicago truncatula* MtPT4 and rice OsPT11 [148–150]. Low-affinity nitrate transporter NPF4.5 plays an important role in mycorrhiza-dependent NO_3^- uptake in rice roots [151]. High-affinity ammonium transporter AMT3;1 functions in mediating the acquisition of mycorrhizal NH_4^+ in maize roots [152]. Tomato high-affinity K^+ transporter *SlHAK10* – which is upregulated by AM infection and is exclusively expressed in arbuscule-containing cells – mediates K^+ uptake through the mycorrhizal pathway [153]. Transcriptome analyses suggest that a putative K^+/H^+ exchanger is upregulated under K^+ deficiency in mycorrhizal roots of *Medicago truncatula* [154]. As expected, some PMA genes are induced by the infection of AMF and located on the periarbuscular membrane in plant roots, such as *OSA8/OsHA1* in rice, *MtHA1* in *M. truncatula*, and *SlHA8* in tomato [155–157]. Knockout of these PMA genes affects the growth of arbuscular mycorrhiza, and impairs the ability of plants to obtain nutrients through symbiosis with AMF, while overexpression of these PMA genes promotes the colonization of AMF in plant roots and enhances the acquisition of P and N [155–157]. Thus, PMA is indispensable for the uptake of nutrients from the interfacial apoplast during the symbiosis (Figure 4).

Modulation of PMA in crop improvement to increase nutrient use efficiency and adaptation to nutrient stresses

In agricultural production, crops have to cope with concentration fluctuation of available nutrients in soils, and nutrient stresses often occur, such as the deficiency of individual nutrients, as well as combined nutrient stresses in soil, such as combined N and P deficiencies, combined P and K deficiencies, and combined P deficiency and Al toxicity [158,159]. It has been found that overexpression of a rice PMA gene *OSA1* promotes the uptake of multiple nutrients and improves nutrient use efficiency [84,160]. Therefore, genetic modulation of PMA genes and/or PMA activity is a potentially effective strategy to improve the acquisition of multiple nutrients and simultaneously enhance the adaptation to nutrient deficiencies in crops, especially in infertile soils (Figure 5). Because PMA is an energy (ATP)-consuming enzyme with multiple physiological functions, if the PMA activity is exorbitantly high, it will lead to negative effects such as excessive energy consumption, growth inhibition, ion toxicity, and abnormality in stomatal closure [161]. Thus, the activity of PMA should be maintained at a suitable level. One of the potential strategies is to drive PMA gene expression using a tissue-specific (such as root-specific) or stress-specific (such as low N-inducible) promoter. Recently, it has been found that activation of PMA expression in arabidopsis roots by grafting a wild-type scion to the rootstock of an *ost2-2D* mutant, which constitutively expresses an active allele of *AHA1* [161], improves nutrient use efficiency and nutrient stress tolerance [162]. It is also a potential strategy to increase nutrient stress resistance and/or nutrient uptake efficiency by grafting a transgenic rootstock with enhanced PMA activity to a non-transgenic scion in horticultural plants and trees (Figure 5). Genome editing of the PMA gene promoter through clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system could also improve PMA activity under normal or nutrient-deficient conditions. Modulating the expression of upstream regulatory factors of PMA – such as transcription factors, 14-3-3 proteins, protein kinases and protein phosphatases – is also a possible strategy to regulate PMA activity. In addition, mutagenesis of certain amino acids (especially at phosphorylation sites) of PMA, possibly by prime editing to modulate PMA activity, could also be used in the genetic improvement of nutrient efficiency in crops (Figure 5).

Concluding remarks and future perspectives

PMA plays pivotal roles in the acquisition of nutrients by generating membrane potential, providing proton motive force, and maintaining cellular pH. However, our understanding of the role and the underlying molecular regulatory mechanism of PMA in plant nutrient acquisition and stress tolerance is still limited. There are still many questions to be answered. Enlarging our understanding of PMA in plant nutrition will be helpful for improving crop nutrient acquisition and utilization

Outstanding questions

How is PMA regulated by nutrient signals at transcriptional and post-translational levels? The associated transcription factors, kinases, or phosphatases regulating PMAs under nutrient stresses have to be identified.

Do tonoplast-localized PMAs, as well as other H^+ pumps (including H^+-PPases and VHAs), also function in nutrient transport? How are they coordinated in nutrient transport and nutrient stress tolerance?

Do PMAs play a role in the uptake and utilization of other nutrients besides N, P, K, and Fe? For example, given that the transport of sulfate and silicate also requires synergistic H^+ influx, PMAs could also be involved in the acquisition of these nutrients.

Do PMAs physically interact with other PM-localized transporters, and how are they coordinated in physiological processes such as nutrient transport?

How can PMAs be genetically modified to increase nutrient use efficiency, enhance nutrient stress tolerance, optimize the balance of nutrients, and/or promote the integration of carbon and nutrient metabolism?

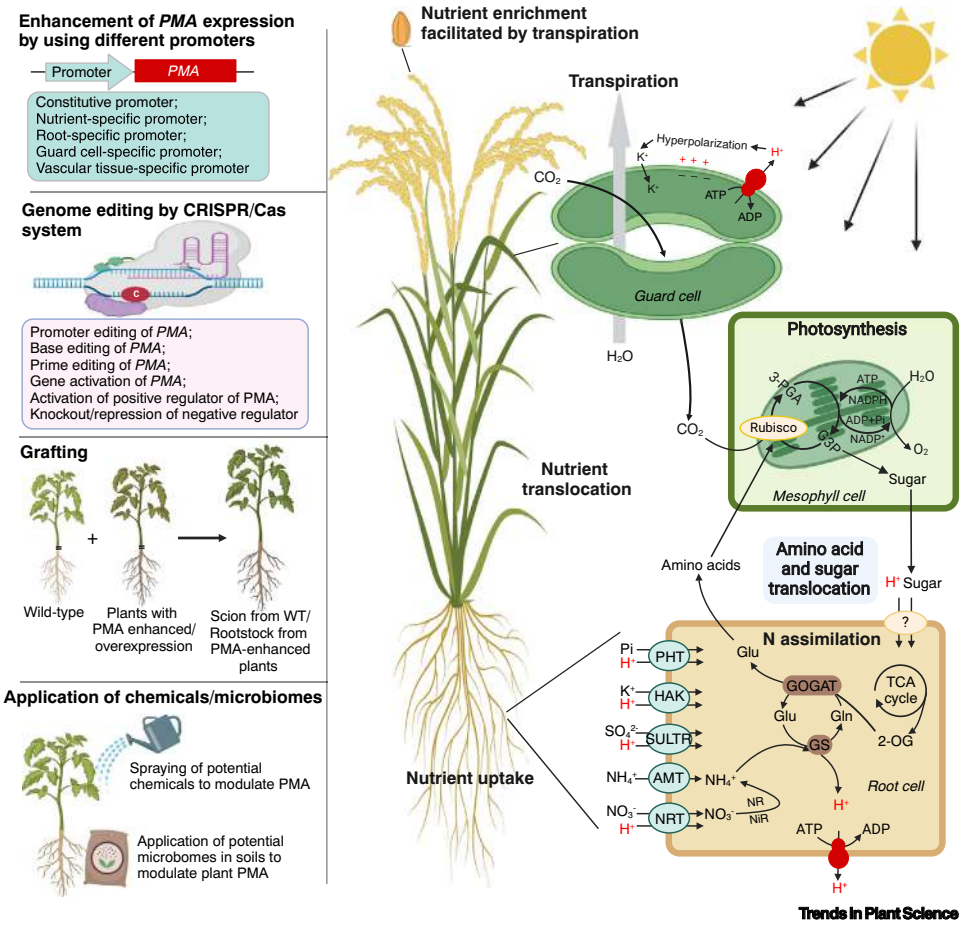

Figure 5. Strategies for modulating plasma membrane H^+-ATPases (PMAs) to improve nutrient use efficiency in crops. Genetic transformation of crops using the expression vector harboring *PMA* genes driven by constitutive, nutrient-specific, or root-specific promoters, and the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system-mediated genome editing of the PMA and its positive or negative regulators, are potential strategies to genetically modulate PMA activity to improve nutrient use efficiency in crops. Grafting by using a PMA-enhanced rootstock, spraying chemicals, or application of microbiomes that could increase plant PMA activity are potential non-transgenic strategies to increase nutrient acquisition and use efficiency in crops. PMA is involved in various physiological processes including nutrient uptake and assimilation, nutrient translocation, amino acid translocation, sugar phloem loading, stomatal movement, and photosynthesis. These physiological processes are closely interconnected. For example, N assimilation provides amino acids for protein biosynthesis in photosynthesis; stomatal opening promotes water transpiration, which could further facilitate nutrient translocation and nutrient enrichment in grains. Thus, it is possible to simultaneously improve nutrient use efficiency, photosynthesis, sugar translocation, and nutrient enrichment by harnessing the expression of PMA. Abbreviations: AMT, ammonium transporter; ERM, extraradical mycelium; HAK, high-affinity potassium transporter; IRM, intraradical mycelium; NiR, nitrite reductase; NR, nitrate reductase; NRT, nitrate transporter; PHT, phosphate transporter. Figure created with BioRender.

efficiency as well as nutrient stress tolerance by modulating PMA activities through biotechnological strategies, which is essential for the development of eco-friendly agriculture.

Carbohydrates from photosynthesis are essential for plant growth as well as for nutrient uptake and assimilation, and there is a synergistic relationship between carbon assimilation and nutrient uptake. Stomatal aperture, which is regulated by PMA, is closely associated with carbon assimilation. Overexpression of *AHA2* in guard cells by using the *GC1* promoter significantly improves stomatal opening, as well as the CO_2 assimilation rate in plants [163]. Recent studies show that

overexpression of *OSA1* promotes photosynthesis and enhances the uptake of N, P, and K, as well as increasing grain yield and nutrient use efficiency in rice [84,160]. Further studies are required to explore the function of PMA in the efficient coordination of carbon assimilation and nutrient metabolism. By modulating PMA in crop improvement, it is possibly to simultaneously improve photosynthesis, sugar translocation, and nutrient use efficiency in crops (Figure 5). Nowadays, elevated atmospheric CO_2 accompanied by global climate change decreases accumulation of macro- as well as micronutrients in plants, especially in cereal crops, and threatens food production and nutritional quality [164]. High CO_2 concentrations induce stomatal closure, and recently this has been demonstrated to be associated with CO_2-mediated dephosphorylation of PMA in guard cells [165]. Therefore, it is a potential strategy to improve nutrient uptake and the accumulation of beneficial elements as well as CO_2 fixation by modulating PMA under the currently elevating atmospheric CO_2 environment and global climate change (see Outstanding questions).

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Declaration of interests

No interests are declared.

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