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REVIEW ARTICLE

Emerging roles of inositol pyrophosphates in signaling plant phosphorus status and phytohormone signaling

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Abbreviations–P, phosphorus; Pi, phosphate; PUpE, phosphate uptake efficiency; PUtE, phosphate utilization efficiency; P1BS, PHR1-binding sequence; InsPs, inositol phosphates; PP-InsPs, inositol pyrophosphates; PA, phytic acid; PSR, phosphate starvation response; PSI genes, phosphate starvation induced genes; PAGE, polyacrylamide gel electrophoresis; IAA, auxin; JA, jasmonic acid; SA, salicylic acid.

ABSTRACT

Phosphorus (P) is an indispensable macronutrient serving a variety of functions in plants. Inositol pyrophosphates (PP-InsPs) nutrient messengers play vital roles in the signaling of P status and plant growth and development. In this review, we summarize (1) the biosynthetic pathway of PP-InsPs and their regulation by plant P status, (2) the effects of PP-InsPs on the function of the SPX-domain containing proteins in signaling plant P status, (3) the effects of inositol pyrophosphates on auxin signaling through TIR1 and on jasmonate signaling through COI1, and (4) the potential crosstalk between P status signaling and phytohormone signaling in plants mediated by inositol pyrophosphates. It is concluded that the interactions between inositol pyrophosphates and their binding proteins are central to plant P status and developmental responses to different P supply.

Keywords: inositol phosphates; inositol pyrophosphates; the SPX-domain containing proteins; TIR1; COI1; phosphorus status; auxin; jasmonic acid

INTRODUCTION

Phosphorus (P) is an indispensable macronutrient for plants (White and Hammond 2008). It is largely present in nucleic acids, phospholipids and phosphorylated metabolites (White and Hammond 2008; Scheible and Rojas-Triana 2015). Although the total amount of P in soil is often abundant, it mainly exists in the form of organic P and insoluble P, while the quantity and concentration of inorganic phosphate (Pi) that can be taken up directly by plants in the soil solution is small (Raghothama and Karthikeyan 2005; White and Hammond 2008; Peret et al. 2011; Lopez-Arredondo et al. 2014; Wang et al. 2018). The application of inorganic Pi fertilizer is the main agronomic method to increase Pi phytoavailability, but only 30-60% of the applied Pi fertilizer is utilized by crops in the year that it is applied (Schachtman et al. 1998; Syers et al. 2008). Excessive application of Pi fertilizers not only increases the cost of agricultural activities, but also increases the flow of P into rivers, lakes, and oceans, causing environmental problems (Scheible and Rojas-Triana 2015).

Through evolution, plants have developed a series of morphological, physiological and molecular mechanisms to improve Pi uptake efficiency (PUpE) and Pi utilization efficiency (PUtE) (White and Hammond 2008; Veneklaas et al. 2012; Wang et al. 2019b; Han et al. 2022b; Wen et al. 2022). Low Pi availability promotes the elongation of lateral roots and formation of root hairs (Lynch 2011; Peret et al. 2011), as well as the establishment of mycorrhizal symbiosis (Lopez-Arredondo et al. 2014; Wen et al. 2022), enabling greater access to the soil volume. The synthesis and secretion of plant acid phosphatases, ribonucleases, and organic acids are induced by low Pi availability, which release Pi from organophosphates, RNA, and Pi-metal ion complexes, respectively (Fang et al. 2009; Du et al. 2022; Wen et al. 2022). Low Pi availability also increases the abundance of Pi transporters to improve Pi uptake by plants (White and Hammond 2008; Lopez-Arredondo et al. 2014). The glycolysis- and oxidative-phosphorylation pathways in plant cells are severely curtailed under low Pi availability, decreasing the P demand of metabolism (Plaxton and Tran 2011) and the phospholipid content of lipid membranes, while the non-phospholipid content is increased, thereby decreasing tissue P requirements (White and Hammond 2008; Sun et al. 2021; Yang et al. 2021). The redistribution of P from old leaves to developing tissues is also enhanced in plants lacking P to maintain photosynthetic capacity (Scheible and Rojas-Triana 2015).

An elaborate signaling network regulates the morphological, physiological and biochemical adaptations to fluctuations in Pi supply, which has been well reviewed (Franco-Zorrilla et al. 2004; Secco et al. 2012; Liu et al. 2014; Gu et al. 2016; Wang and Liu 2018; Pan et al. 2019). In particular, fluctuations in Pi supply alter hormone biosynthesis, transport, and signaling, which have been implicated in plant acclimation to vagaries in Pi availability (Chiou and Lin 2011). Although substantial insights into the molecular biology of the regulatory networks regulating plant P status and phytohormone signaling have been obtained, exact knowledge is limited on how plants sense external Pi availability and to evoke proper intracellular responses.

This review summarizes the biosynthetic pathway of inositol (pyro)phosphates and their regulation by plant P status, and the functions of the SPX-domain containing proteins that bind inositol (pyro)phosphates in signaling plant P status and co-receptors in phytohormone response pathways. It highlights the possible new roles of inositol (pyro)phosphates in mediating plant P status, and the crosstalk between P status and phytohormone signaling.

BIOSYNTHESIS OF INOSITOL PYROPHOSPHATES IN PLANTS

Myo-, D-*chiro*-, L-*chiro*-, *muco*-, *scyllo*-, and *neo*- inositol are naturally occurring isomers, of which *myo*-inositol is the most abundant form (Michell 2008; Pani et al. 2020). The *myo*-inositol isomer is the skeleton for inositol phosphates (InsPs), which are a series of phosphorylated inositol metabolites synthesized by the multi-step phosphorylation of *myo*-inositol. Phytic acid (PA) is the fully phosphorylated *myo*-inositol (Fig. 1). PA is synthesized via lipid-dependent or lipid-independent pathways (Wang et al. 2022). Both lipid-dependent and lipid-independent pathways rely on phosphorylation of several kinases to produce $InsP_5$, which is subsequently phosphorylated by IPK1 (inositol pentakisphosphate 2-kinase) to yield PA (Wang et al. 2022). PA can be (1) transported via a multidrug-resistance-associated protein (MRP) to protein storage vacuoles (PSV) where it is accumulated into globoids in the form of PA salts (Otegui et al. 2002; Shi et al. 2007; Krishnan 2008; Regvar et al. 2011); (2) decomposed by phytase to release Pi, inositol and associated cations; or (3) used as a precursor to be further phosphorylated into inositol pyrophosphates (PP-InsPs). PA plays a central role in the synthesis of PP-InsPs (Fig. 1).

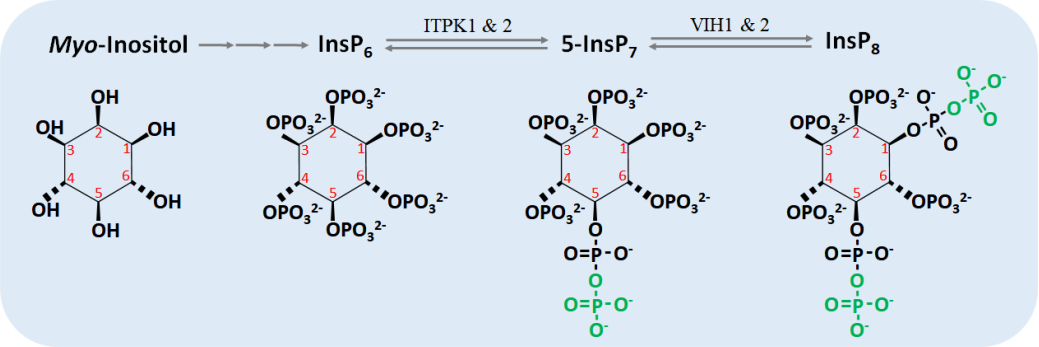

Fig. 1 The chemical structure of *myo*-inositol, phytic acid ($InsP_6$), and inositol pyrophosphates (5-$InsP_7$ and $InsP_8$). ITPK1 & 2, Inositol tetrakisphosphate kinase 1 & 2; VIH1 & 2, Diphosphoinositol pentakisphosphate kinase 1 & 2.

The biosynthesis of PP-InsPs in plants is mainly catalyzed by two types of bifunctional enzymes, inositol tetrakisphosphate kinase (ITPK) and diphosphoinositol pentakisphosphate kinase (VIH) (Fig. 1). In *Arabidopsis*, PA can be phosphorylated at the 5-position phosphate group by ITPK1 to yield $InsP_7$, and kinetic analysis revealed that ITPK1 exhibits an extremely high K_M for ATP of approximately 520 μM (Laha et al. 2019; Riemer et al. 2021). ITPK1 not only phosphorylates $InsP_6$ to generate $InsP_7$, but also mediates the decomposition of 5-$InsP_7$ (Riemer et al. 2021). An interesting question regarding bifunctional enzyme is how ITPK1 catalyzes the reverse reaction of the kinase activity as there is no phosphatase domain within ITPK1 protein. Kinetic analyses demonstrate that ITPK1 has comparable K_M values for ATP and ADP, and ITPK1 can shift its activity to an ADP-phosphotransferase that can transfer Pi from the 5-position phosphate group of 5-$InsP_7$ to ADP at low ATP/ADP ratio conditions (Riemer et al. 2021). Additionally, there was no ADP-phosphotransferase activity of ITPK1 with any other $InsP_7$ isomer, suggesting a substrate specificity for the reaction. Collectively, Pi-dependent changes in ATP concentration and the ATP/ADP ratio may ultimately determine the production of 5-$InsP_7$ by shifting ITPK1-mediated $InsP_6$ kinase and ADP-phosphotransferase activities (Riemer et al. 2021).

$InsP_7$ is phosphorylated by VIH1 and VIH2 to generate $InsP_8$. The products of $InsP_8$ are likely 1,5-$InsP_8$ and its enantiomeric isomer 3,5-$InsP_8$, but the enantiomer identity has not been resolved (Fig. 1; Dong et al. 2019; Zhu et al. 2019; Laha et al. 2019). Both VIH1 and VIH2 are bifunctional enzymes that synthesize and decompose $InsP_8$. Although VIHs contain both kinase and phosphatase domains, only mutating the kinase active center, but not the phosphatase active center, leads to constitutive P starvation responses (PSR) and P accumulation (Dong et al. 2019). *In vitro*

experiments show that Mg^{2+}-ATP concentrations control the relative kinase and phosphatase activities of VIH1 and VIH2 and that Pi inhibits the phosphatase activity of the enzyme (Zhu et al. 2019). Thus, VIH1 and VIH2 regulate the production of $InsP_8$ depending on intracellular ATP and Pi concentrations, which vary with external P supply (Zhu et al. 2019).

INOSITOL PYROPHOSPHATES SIGNALING OF PLANT PHOSPHORUS STATUS

Inositol pyrophosphates control phosphorus status

The SPX (SYG1, Pho81 and XPR1) domain (PfamPF03105) were named after a homologous sequence shared by yeast SYG1, PHO81 and human XPR1 (Secco et al. 2012). In plants, the SPX domain-containing proteins can be divided into four subfamilies: SPX proteins, SPX-EXS (EXS, named after the yeast ERD1, the human XPR1 and the yeast SYG1) proteins, SPX-MFS (MFS, the major facilitator superfamily) proteins, and SPX-RING (RING, the really interesting new gene) proteins (Secco et al. 2012). The SPX domain in the SPX domain-containing proteins contains a putative binding site for PP-InsPs, which could play a key role in signaling plant P status and affecting the regulation of plant P as an intracellular sensor in plants (Fig. 2A, Fig. 2B, Table1). Since many InsPs and PP-InsPs exist in plant cells, one question is which molecules are involved in signaling plant P status and affecting internal P concentrations. Using titanium dioxide (TiO2)-based pull-down followed by PAGE, and capillary electrophoresis electrospray ionization mass spectrometry (CE-ESI-MS), different species of $InsP_6$, $InsP_7$, and $InsP_8$ were quantified under P-sufficient and -deficient conditions (Riemer et al. 2021). In *Arabidopsis thaliana*, the concentrations of $InsP_6$, $InsP_7$ and $InsP_8$ decreased significantly upon P deficiency. After resupplying P, the increase of $InsP_7$ and $InsP_8$ concentrations were significantly larger than that of $InsP_6$ concentration, with the concentration of $InsP_8$ increasing approximately 100-fold, greatly exceeding the concentration detected in plants grown continuously with an adequate P supply (Riemer et al. 2021). $InsP_7$ and $InsP_8$ are most sensitive to fluctuations in external P supply, suggesting that they may be intracellular signaling molecules allowing plants to respond to external P supply. Similar responses are also observed in rice and *Physcomitrium patens*, suggesting that the response to P availability in the biosynthesis of $InsP_7$ and $InsP_8$ is evolutionarily conserved in plant kingdom (Riemer et al. 2021).

Similar to IPK1, ITPK1 is widely distributed at the tissue level, localized to the cytoplasm and nucleus, and the expression of *ITPK1* is not induced by P deficiency (Kuo et al. 2018). The *ipk1-1*/*itpk1* double mutant exhibits more severe growth reduction than single mutants and plants that proceeded to the reproductive stage have aborted seeds (Kuo et al. 2018). Although tissue P concentrations are greater in the *ipk1-1*/*itpk1* double mutant than in single mutants, by 50~70%, this might be attributed to the relative 50~80% reduction in fresh weight of the former. The expression of phosphate starvation induced (PSI) genes in *ipk1-1*/*itpk1* double and single mutants was comparable, indicating that ITPK1 and IPK1 are in a common response pathway to plant P status (Kuo et al. 2018).

In vitro assays have shown that ITPK2 also has $InsP_6$ kinase activity, however, only the disruption of ITPK1, but not of ITPK2, results in growth defects and constitutive P overaccumulation (Riemer et al. 2021). Concentrations of 5-$InsP_7$, $InsP_8$, and other inositol (pyro)phosphates in the *itpk2* mutant were similar to wild-type (Laha et al. 2019; Riemer et al. 2021). Despite the different phenotypes of the *itpk2* and *itpk1* mutants, the growth reduction and P hyperaccumulation in *itpk1* are not as severe as *vih1*/*vih2* mutants, which is unable to catalyze the conversion of $InsP_7$ to $InsP_8$ (Fig. 1), suggesting partial functional redundancy of *ITPK2* and *ITPK1* (Dong et al. 2019; Zhu et al. 2019; Riemer et al. 2021). However, when grown in P-sufficient conditions, the *itpk1*/*itpk2* double mutant exhibits severe growth reduction, and its shoot P concentration was approximately 3.5-fold and 2.1-fold higher than wild-type and *itpk1*, respectively, suggesting that ITPK2 plays a relatively minor role in signaling plant P status in the presence of a functional ITPK1 (Riemer et al. 2021).

Among a series of mutants in the PA and PP-InsPs biosynthesis pathways, only *ipk1-1* and *itpk1* show significant increases in shoot P concentration from seedlings to the mature plant (Kuo et al. 2018). PHOSPHATE2 (PHO2) is an ubiquitin-conjugating enzyme (UBC24) that ubiquinates the SPX-EXS Pi transporter PHOSPHATE1 (PHO1), resulting in its degradation and a decrease in xylem Pi loading (Liu et al. 2012). Compared with *ipk1-1* and *pho2* single mutants, the *ipk1-1*/*pho2* double mutant showed additive shoot P accumulation, suggesting that P hyperaccumulation in the *ipk1-1* mutant was mainly independent of the PHO2 regulatory pathway (Kuo et al. 2014). In comparison with the *ipk1-1* single mutant, the P concentration and the expression of several genes regulated directly by the transcription factor PHOSPHATE

STARVATION RESPONSE1 (PHR1) and PHR1-like 1 (PHL1) in the *ipk1-1*/*phr1* double and *ipk1-1*/*phr1*/*phl1* triple mutants were significantly decreased, but they were still greater than those in *phr1* and *phr1*/*phl1* mutants, respectively, indicating that PHR1 (PHL1) plays a partial role in upregulating the expression of PSI genes and P hyperaccumulation in the *ipk1-1* mutant (Kuo et al. 2014). Similarly, the shoot P concentration of the *itpk1*/*pho2* double mutant was approximately twice that of *itpk1* and *pho2* mutants, suggesting that P hyperaccumulation in the *itpk1* mutant is also independent of the PHO2 regulatory pathway (Riemer et al. 2021). Although *phr1*/*itpk1* and *phr1*/*phl1*/*itpk1* mutants accumulate more P than *phl1* and *phr1*/*phl1* mutants, respectively, the relative increments are smaller than in the presence of functional PHR1 and PHL1, suggesting that PHR1 (PHL1) is tightly linked to ITPK1-mediated regulation of plant P status (Riemer et al. 2021).

The growth of *vih2*/*itpk1* is slower than *itpk1*, and shoot P accumulation in *vih2*/*itpk1* is greater by about 27%, suggesting that VIH2 and ITPK1 are located in the same regulatory pathway affecting plant P status (Riemer et al. 2021). The concentration of $InsP_8$ was decreased in *vih2* and undetectable in the *vih1*/*vih2* double mutants (Dong et al. 2019). Although *vih1* and *vih2* single mutants have similar P concentrations to wild-type plants, *vih1*/*vih2* double mutants have severely restricted growth and significantly increased P accumulation, indicating that VIH1 and VIH2 are functionally redundant in Arabidopsis (Dong et al. 2019; Zhu et al. 2019). Knockout of PHR1 in the *vih1*/*vih2* mutant partially complements its phenotype, suggesting that VIH1/VIH2 functions in signaling and regulating plant P status (Dong et al. 2019; Zhu et al. 2019).

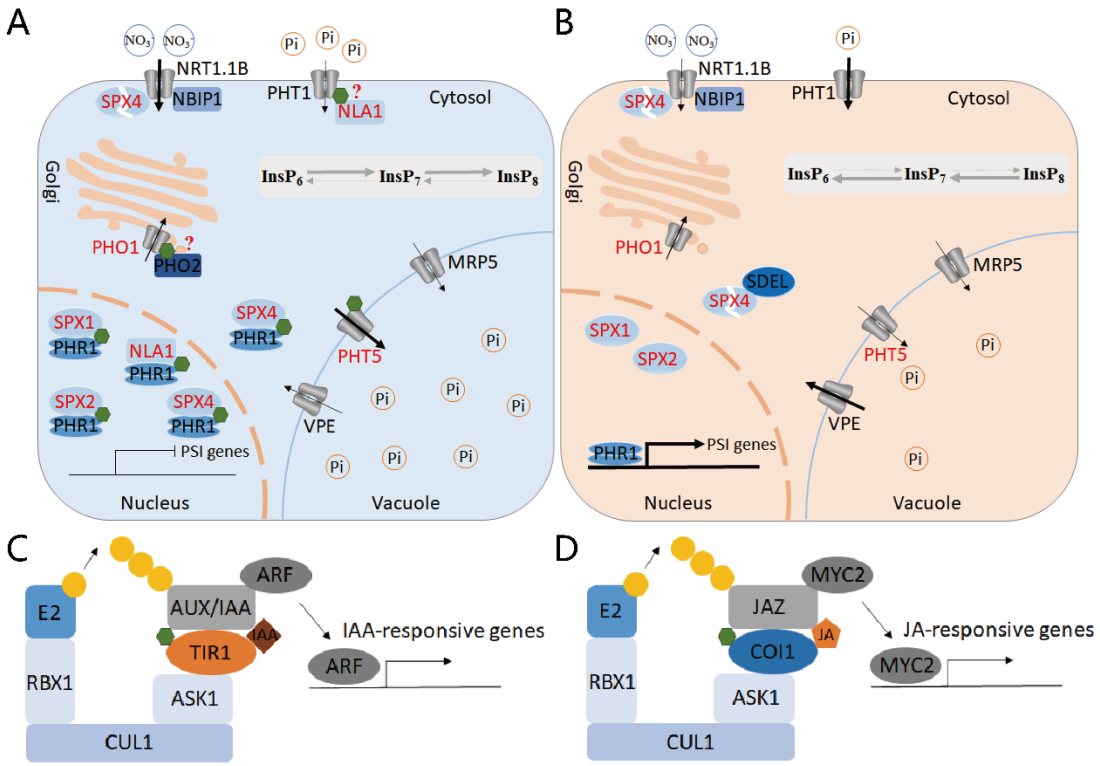

Fig. 2 Inositol pyrophosphates binding proteins in plant cells and their function.

(A) Under P sufficient conditions, Pi stimulates the synthesis of PP-InsPs, and PP-InsPs involved in signaling plant P status by regulating the function of the SPX domain-containing proteins. (B) Under P deficient conditions, PP-InsPs are hydrolyzed and their actions on the SPX-domain containing proteins are abolished. (C) InsPs and PP-InsPs involved in auxin signaling by regulating the function of TIR1 in plants. (D) InsPs and PP-InsPs involved in jasmonic acid signaling by regulating the function of COI1 in plants. Green hexagons indicate InsPs or PP-InsPs, and yellow circle indicates ubiquitin.

Table 1 Inositol pyrophosphates binding proteins in plants and their functions

PP-InsPs binding proteins	Interaction protein	InsPs/PP-InsPs dependency	Consequence of interaction	References
SPX (AtSPX1~AtSPX4; OsSPX1~OsSPX6)	AtPHR1/OsPHR2	Yes ($InsP_{6/7/8}$)	Block PHR-mediated transcription activation of PSI genes	Puga et al. 2014; Wang et al. 2014b; Wild et al. 2016; Dong et al. 2019; Zhou et al. 2021; Guan et al. 2022
	OsSDEL1/2	No	Degrade SPX4 to release PHR	Ruan et al. 2019
	OsNRT1.1B	No	Recruit NBIP1 to degrade SPX4	Hu et al. 2019
	OsNBIP1	No	Degrade SPX4 to release PHR and NLP	Hu et al. 2019
	OsNLP3	No	Block NLP-mediated transcription activation of nitrate responsive genes	Hu et al. 2019
	OsRLI1	Unknown	Inhibit RLI1 to regulate leaf inclination	Ruan et al. 2018; Zhang et al. 2021

	AtPAP1	Yes ($InsP_6$)	Block PAP1-mediated transcription activation of anthocyanin biosynthesis	He et al. 2020b
	OsbHLH6	Unknown	Block the effect of SPX4 on PHR2	He et al. 2021a
SPX-EXS (AtPHO1; AtPHO1;H1~H10; OsPHO1;1~1;3)	AtPHO2	Unknown	Degrade AtPHO1 to reduce Pi loading	Liu et al. 2012
SPX-MFS (AtSPX-MFS1~3; OsSPX-MFS1~4; FaVPT1)	Within VPT1 protein (i.e., SPX domain and MFS domain)	Yes ($InsP_8$)	Activate the transport activity of VPT1	Luan et al. 2022
SPX-RING (AtNLA1~AtNLA2; OsNLA1~OsNLA2)	AtPHT1	Unknown	Degrade AtPHT1 to reduce Pi uptake	Kant et al. 2011; Lin et al. 2013
	OsPHT1	Unknown	Degrade OsPHT1 to reduce Pi uptake	Yue et al. 2017; Yang et al. 2020
	AtNRT1.7	Unknown	Degrade NRT1;7 to reduce nitrate redistribution	Liu et al. 2017
	AtORE1	Unknown	Degrade ORE1 to alleviate leaf senescence	Park et al. 2018
	AtPHR1	Yes ($InsP_8$)	Degrade PHR1 to block transcription activation of PSI genes	Park et al. 2022
TIR1	IAA7	Yes ($InsP_{6/7}$)	Degrade IAAs to release ARFs	Tan et al. 2007; Calderon Villalobos et al. 2012; Laha et al. 2022
COI1	JAZ	Yes ($InsP_8$)	Degrade JAZs to release MYCs	Sheard et al. 2010; Mosblech et al. 2011; Laha et al. 2015; Laha et al. 2016

SPX protein subfamily

There are four members in *Arabidopsis* SPX protein subfamily, known as *AtSPX1~AtSPX4* (Duan et al. 2008). Except for *AtSPX4*, the expression of other members is induced by P deficiency, among which *AtSPX1* and *AtSPX3* are strongly induced while *AtSPX2* is only slightly induced (Duan et al. 2008). The *spx1/spx2* double mutant exhibits an increased activity of PHR1 in plants grown in P-sufficient conditions but only a minor alteration of PHR1 activity in P-deficient plants, indicating that the inhibitory effect on PHR1 of SPX1 and SPX2 is cellular Pi-dependent (Puga et al. 2014). The interaction of SPX1 and PHR1 is compromised under P-sufficient conditions in the *vih1*/*vih2* mutant, leading to a constitutive activation of PSI genes. Furthermore, isothermal titration calorimetry shows that $InsP_8$ binds directly to SPX1 proteins and co-immunoprecipitation demonstrates that the interaction of SPX1 and PHR1 is Pi- and $InsP_8$-dependent (Dong et al. 2019). Recently, it was reported that the KHR motif ($PHR1^{K325}$, $PHR1^{H328}$, and $PHR1^{R335}$) at the surface of the coiled-coil (CC) domain of AtPHR1 is essential for

its interaction with AtSPX1 (Ried et al. 2021). The Pi-$InsP_8$-SPX1-PHR1 working model indicates that $InsP_8$ is an intracellular signaling molecule which is sensitive to Pi concentration, and SPX1 suppresses the activities of PHR1 in an $InsP_8$-dependent manner as an intracellular sensor (Fig. 2 A and B).

AtSPX4 functions as a repressor not only in PHR1-dependent but also in PHR1-independent pathways in P-sufficient plants. Gene regulatory network analyses revealed that SPX4 interacts with several regulators of shoot development, such as SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1) and ARABIDOPSIS NAC DOMAIN CONTAINING PROTEIN55 (ANAC055) (Osorio et al. 2019). SPX4 acts as a regulator not only in signaling P status, but also in transmitting the P deficiency signal to anthocyanin biosynthesis. The MYB transcription factor PRODUCTION OF ANTHOCYANIN PIGMENTS1 (PAP1) controls the synthesis of anthocyanin by activating the expression of genes encoding the proteins in the synthesis pathway (He et al. 2021b). SPX4 interacts with PAP1 to inhibit the binding of PAP1 to the promoter of its downstream genes in a PP-InsPs -dependent manner under P-sufficient conditions, conversely, in the absence of PP-InsPs under P-deficient conditions, the interaction between SPX4 and PAP1 is compromised, and PAP1 is released to initiate anthocyanin biosynthesis (He et al. 2021b).

In rice, there are six members in SPX protein subfamily, namely OsSPX1~OsSPX6 (Zhong et al. 2018). Except for *OsSPX4*, which is not responsive to P deficiency, the other five *SPX* genes are all induced significantly after 7 days of P deficiency (Zhong et al. 2018). Rice transcription factor PHR2 is composed of transcriptional activation domain, MYB domain responsible for DNA binding, and CC domain responsible for dimerization, from N- to C-terminal (Wang et al. 2014b). Nuclear-localized OsSPX1 and OsSPX2 regulate P status by inhibiting the activity of PHR2 in a Pi-dependent manner (Wang et al. 2014b). Unexpectedly, PHR2 binds to P1BS elements in dimerized form *in vivo* to activate the expression of PSI genes in P-deficient conditions (Zhou et al. 2021). When two OsSPX1 proteins approach a dimerized PHR2 in P-sufficient conditions, their helix α1 will be sterically hindered, resulting in the inability of the OsSPX1s bind to the PHR2 dimer (Zhou et al. 2021). Upon binding PP-InsPs, an allosteric effect is produced so that OsSPX1s can disrupt PHR2 dimers and form a SPX1-PHR2 complex in a 1:1 ratio. In this instance, the DNA binding activities of PHR2 will be sterically blocked (Zhou et al. 2021). Similarly, in the OsSPX2-$InsP_6$-PHR2 complex, the $InsP_6$-binding OsSPX2 proteins assemble into a dimer and

binds two molecules of PHR2, making the MYB domain of PHR2 severely allosteric turned and unable to bind DNA molecules (Guan et al. 2022).

Rice REGULATOR OF LEAF INCLINATION1 (OsRLI1) is a transcription factor positively regulating leaf inclination by affecting lamina joint cell elongation in rice (Zhang et al. 2021). OsRLI1 directly activates the downstream genes OsBU1 and OsBU1-LIKE1 COMPLEX1 to regulate elongation of the lamina joint cells (Ruan et al. 2018). OsSPX1 protein interacts directly with OsRLI1, which could prevent OsRLI1 binding to the promoter of its downstream genes. In this way, OsSPX1 can also regulate leaf inclination by inhibiting the transcriptional activity of OsRLI1 in rice, whether this process is dependent on PP-InsPs remains to be further investigated (Ruan et al. 2018).

OsSPX4 can interact with OsPHR2 to prevent the latter from entering the nucleus or binding to P1BS elements in downstream genes, thereby regulating P status (Lv et al. 2014). Although OsSPX4 does not respond to changes in external P availabilities at the transcriptional level, its protein stability is reduced under P-deficient conditions (Lv et al. 2014). RING-type E3 ubiquitin ligases OsSDEL1 and OsSDEL2 induced by P deficiency are involved in the degradation of OsSPX4 protein, resulting in the release of OsPHR2, allowing plants to adapt to P deficiency (Ruan et al. 2019). Furthermore, OsPHR2 competes with OsSDELs by interacting with OsSPX4 under P-sufficient conditions, which protects OsSPX4 from ubiquitination and degradation (Ruan et al. 2019). *In vitro* assays provide a more detailed working model of PP-InsPs-SDELs-SPX4-PHR2 complex in rice (Ruan et al. 2019). The presence of PP-InsPs promotes the interaction of OsSPX4 and OsPHR2, which prevents OsSDELs from interacting with OsSPX4 and mediating its degradation under P-sufficient conditions, reversely, the dissociation of the OsSPX4-OsPHR2 in the absence of PP-InsPs releases OsSPX4 to OsSDELs, leading to ubiquitination and degradation of OsSPX4 under P-deficient conditions (Ruan et al. 2019).

Nuclear- and cytoplasm-localized OsbHLH6 exclusively interacts with OsSPX4 but not with other OsSPX proteins, moreover, OsbHLH6 has higher binding affinity with OsSPX4 than OsPHR2. Therefore, OsbHLH6 can alleviate the blocking effect of OsSPX4 on OsPHR2 (He et al. 2021a). The interaction between OsbHLH6 and OsSPX4 mainly occurs under P-sufficient conditions, however, it remains unknown whether the interaction is PP-InsPs-dependent. Under

nitrate-sufficient conditions, nitrate perception strengthens the interaction of OsNRT1.1B and OsSPX4, and OsNRT1.1B interacting protein 1 (OsNBIP1) is recruited to degrade OsSPX4, therefore releasing OsPHR2 and OsNLP3 to promote Pi and nitrate acquisition; while under low nitrate conditions, OsSPX4 interacts with OsPHR2 and OsNLP3 and inhibits the function of OsPHR2 and OsNLP3 in P and nitrate signaling and regulation (Hu et al. 2019).

OsSPX3 and OsSPX5 redundantly regulate plant P status, and genetic analysis indicates that both are repressors of OsPHR2 (Shi et al. 2014). OsSPX6 is localized in cytoplasm and nucleus The interaction of OsSPX6 with OsPHR2 blocks the translocation of OsPHR2 from cytoplasm into the nucleus, and inhibits OsPHR2 binding to the P1BS elements in downstream genes. Thus, OsSPX6 negatively regulates the PSR through suppression of PHR2 (Zhong et al. 2018). In addition, SPX proteins in other species are also involved in plant P status through similar mechanisms, such as GmSPX1 and GmSPX3 in soybean, BnaSPX1 in rapeseed, and MtSPX1 and MtSPX3 in *Medicago truncatula* (Yao et al. 2014; Zhang et al. 2016; Du et al. 2017; Wang et al. 2021).

SPX-EXS protein subfamily

PHOSPHATE1 (PHO1), identified by map-based cloning, shows very low homology to H^+-Pi co-transporters, belonging to a new class of ion transporters in plants (Hamburger et al. 2002). The *PHO1* family has 11 members in the Arabidopsis genome, namely *PHO1* and *PHO1;H1~PHO1;H10 (PHO1 homologs)*, most of which are expressed in the vascular tissues of roots, stems, leaves and flowers (Wang et al. 2004). PHO1 and PHO1;H1 are responsible for the loading of Pi from root epidermal cells and cortical cells to xylem vessels (Stefanovic et al. 2007). Loss of PHO1 function can hinder the long-distance transport of Pi from roots to shoots, resulting in the decline of Pi concentrations in the shoot (Stefanovic et al. 2007). There are three homologous genes of *PHO1* in rice, known as *OsPHO1;1*, *OsPHO1;2*, and *OsPHO1;3*, among which OsPHO1;1 and OsPHO1;2 are located in the plasma membrane and mainly expressed in node I, being responsible for the transportation of Pi to grains (Che et al. 2020; Chiou 2020; Ma et al. 2021). Additionally, OsPHO1;2 is also responsible for the long-distance transport of Pi from roots to shoots in rice (Secco et al. 2010). It is worth noting that the regulation of expression of PHO1 occurs at different levels in Arabidopsis. Firstly, there are W-box cis-acting elements in the promoter region of *PHO1*, to which transcription factors WRKY6 and WRKY42 can bind to

inhibit the expression of *PHO1* (Chen et al. al 2009; Su et al. 2015). Secondly, the ubiquitin-conjugating enzyme PHO2 (UBC24) is involved in the ubiquitination of PHO1, resulting in the degradation of PHO1 protein (Liu et al. 2012). Finally, there is an upstream open reading frame (uORF) in the 5' untranslated region of *PHO1*, which can also regulate the protein abundance of PHO1 (Reis et al. 2020).

Pi efflux in human cells is highly dependent on Xenotropic and Polytropic Retrovirus Receptor 1 (XPR1) (Wilson et al. 2019). Isothermal titration calorimetry shows that $InsP_8$ has a very high affinity for the XPR1 protein (Kd=180 nM), and diphosphoinositol pentakisphosphate kinases (PPIP5Ks) mutant cell lines have reduced Pi efflux, while the XPR1 mutant cell lines exhibit a similar phenotype (Li et al. 2020). By mutating PPIP5Ks or adding an inhibitor of inositol hexakisphosphate kinases (IP6Ks), intracellular synthesis of $InsP_8$ can be reduced, thereby inhibiting XPR1-mediated Pi efflux in human cells (Wilson et al. 2019; Li et al. 2020). In *Arabidopsis thaliana*, topological analysis reveals that the N-terminus of the PHO1 protein contains an SPX domain, followed by four transmembrane motifs and an EXS domain (Wege et al. 2016). The SPX domain of PHO1 contains no transmembrane motif and is located in the cytoplasmic side of the cell, providing a putative anchor site to be regulated (Wege et al. 2016). Using a tobacco transient expression system, it was found that the EXS domain of PHO1 is necessary for its Pi transport activity and subcellular localization, but the EXS domain alone cannot transport Pi. Expression of the EXS domain in the *pho1* background rescues the shoot growth defect, while the P concentration remains the same as in *pho1* mutant, suggesting that the SPX domain is indispensable for a functional PHO1 (Wege et al. 2016). Although the mutation of the PP-InsPs binding site in the Arabidopsis PHO1 protein did not affect its subcellular localization, the mutated PHO1 proteins driven by the native promoter are unable to rescue the reduced shoot P concentration of the *pho1* mutant, suggesting that the binding of PP-InsPs is also critical for a functional PHO1 protein (Wild et al. 2016). AtPHO1 was previously identified as a Pi efflux transporter (Arpat et al. 2012; Vogiatzaki et al. 2017), but OsPHO1;1 and OsPHO1;2 were recently identified as Pi influx transporters (Che et al. 2020). The influx activity of PHO1 cannot explain its prominent role in the xylem loading, which requires efflux activity to move Pi out of cells (Stefanovic et al. 2007). Alternatively, it cannot be ruled out that PHO1 is able to mediate bi-directional transport of Pi. The N-terminal part of PHO1, which contains the SPX domain, is

required for the recognition, interaction, and subsequent ubiquitination by PHO2 (Liu et al. 2012). There exists a possibility that the transport direction/activity of PHO1 and the interaction between PHO1 and PHO2 are controlled finely by the concentration of PP-InsPs in plant cells.

SPX-MFS protein subfamily

Plant vacuoles are the main organelle for storing Pi, and vacuole Pi transporter (VPT), also known as SPX-MFS or PHT5, mediates Pi transport between cytosol and vacuole (Yang et al. 2017). The PHT5 family in Arabidopsis includes three members, known as AtPHT5;1, AtPHT5;2 and AtPHT5;3, of which AtPHT5;1 plays a major role in Pi accumulation (Liu et al. 2015; Liu et al. 2016). The SPX-MFS family in rice includes four members, namely OsSPX-MFS1, OsSPX-MFS2 OsSPX-MFS3 and OsSPX-MFS4, among which *OsSPX-MFS1* and *OsSPX-MFS3* are downregulated under P deficiency, whereas *OsSPX-MFS2* is induced (Wang et al. 2012). All the OsSPX-MFS proteins transport Pi from the cytosol to the vacuole, among which OsSPX-MFS3 plays dominant role while OsSPX-MFS2 has the weakest function (Lin et al. 2010; Wang et al. 2015; Xu et al. 2019; Guo et al. 2022). Recently, we identified two vacuolar Pi influx transporters in *B. napus*, and revealed the distinct and conserved roles of BnaPHT5;1bs in cellular Pi status in this plant species (Han et al. 2022a).

Yeast VTC (Vacuolar Transporter Chaperone) is a type of inorganic polyphosphate (polyP) polymerase localized on the tonoplast (Gerasimaite et al. 2017). 5-PP-$InsP_5$ bind specifically to the SPX domain of the VTC protein and acts as the main activator of intracellular VTC, indicating that the SPX domain may integrate PP-InsPs to adapt to cytoplasmic Pi levels under different metabolic conditions (Gerasimaite et al. 2017). When PP-InsPs is binding to the SPX domain within the VTC protein, the catalytic polymerase domain at the entrance of the trans-membrane channel is oriented, both activating the enzyme and coupling polyP synthesis and membrane translocation (Guan et al. 2023). Rice OsSPX-MFS1, OsSPX-MFS2 and OsSPX-MFS3 localize to the tonoplast, and their truncated proteins ΔMFS1, ΔMFS2 and ΔMFS3 with the SPX domain deleted still localized to the tonoplast, suggesting that the transmembrane domain and C-terminal motif are critical for the localization of SPX-MFSs, while the SPX domain probably plays a regulatory role (Wang et al. 2015). The SPX domain of the strawberry FaVPT1 protein shows a high affinity for $InsP_6$ (Kd=3.5 μM), moreover, the SPX-MFS family proteins share highly conserved PP-InsPs binding sites, suggesting that PP-InsPs may also act on SPX-MFS proteins to

control intracellular P homeostasis (Secco et al. 2012; Huang et al. 2019). The auto-inhibitory domain in the VPT1 protein suppresses its transport activity under P deficient conditions. However, under P sufficient conditions activity of VPT1 is activated to transport excess Pi into vacuole upon binding of $InsP_8$ through the SPX domain (Luan et al. 2022).

SPX-RING protein subfamily

Proteins containing the RING domain generally possess ubiquitin ligase (E3) activity, which can transfer ubiquitin from ubiquitin-conjugating enzyme (E2) to specific substrate proteins (Kraft et al. 2005; Stone et al. 2005). In the Arabidopsis and rice genomes, there are only two genes encoding proteins containing both SPX and RING domains, namely NLA1 and NLA2 (Secco et al. 2012; Jung et al. 2018). Screening of 200 T-DNA insertion lines identified a line that failed to develop the essential adaptive responses to low nitrogen conditions, and senesced earlier and more rapidly than wild type under nitrogen deficiency, so it was named NITROGEN LIMITATION ADAPATATION1 (NLA1) (Peng et al. 2007). Two suppressors of *nla1* (*nla1*-Suppressor1 and *nla1*-Suppressor2) were identified by genetic approaches, both of which can rescue the phenotype of *nla1* mutants failing to adapt to nitrogen deficiency. It was found that the two suppressors were *PHF1* and *PHT1;1* mutations after map-based cloning, moreover, the *nla1*/*phf1* or *nla1*/*pht1;1* double mutant can also restore the phenotype of *nla1* (Kant et al. 2011). NLA1 co-localizes with PHT1;1 and PHT1;4 in the plasma membrane, and NLA1 regulates P status by mediating the ubiquitination and degradation of PHT1;1 and PHT1;4 in Arabidopsis (Lin et al. 2013). OsNLA1 also controls P status by ubiquitinating several OsPHT1s in rice, notably, the main difference between two species is that *AtNLA1* is regulated by the microRNA *miR827* at the post-transcriptional level, while *OsNLA1* is not regulated in this manner (Yue et al. 2017; Yang et al. 2020). Interestingly, the phenotype of *nla1* mutant whilst failing to adapt to low nitrogen conditions can not only attribute to Pi toxicity, but also excessive nitrogen transfer from old leaves to new leaves under nitrate deficiency. NLA1 mediates the ubiquitination and degradation of nitrate transporter NRT1;7, and regulates the redistribution of nitrate from source to sink in plants under low nitrogen conditions (Liu et al. 2017). ORE1 is a core transcription factor that controls leaf senescence under nitrate deficient conditions, and NLA1 also regulates leaf senescence under nitrogen limitation by mediating ORE1 ubiquitination and degradation (Park et al. 2018).

Sequence alignment shows that the binding site of PP-InsPs in NLA1 is highly conserved

(Secco et al. 2012). Furthermore, both mutation in genes related to PP-InsPs synthesis (e.g., *IPK1*, *ITPK1*, and *VIHs*) and *NLA1* leads to P overaccumulation (Lin et al. 2013; Kuo et al. 2018; Dong et al. 2019). It was shown that the SPX domain of NLA1 not only interacts with the Pi transporter PHT1s, but also with the nitrate transporter NRT1;7 (Lin et al. 2013; Liu et al. 2017). Although it was revealed that NLA1 mediates the ubiquitination of PHR1 in a PP-InsPs-dependent manner (Park et al. 2022), it remains largely unknown whether PP-InsPs affect the NLA1-PHT1s module to control Pi uptake, or on the NLA1-NRT1;7 module to regulate nitrate status in plants. The SPX domain of NLA2 also contains a conserved PP-InsPs binding site, suggesting that NLA2 may also work coordinately with PP-InsPs to control P status in plants (Secco et al. 2012; Jung et al. 2018).

INOSITOL (PYRO)PHOSPHATES MEDIATED AUXIN SIGNALING PATHWAY

Inositol (pyro)phosphates and auxin co-receptor TIR1

Auxin is widely involved in plant growth, development, and stress adaptation (Salehin et al. 2015). There are four types of auxins derived from plants, of which indole-3-acetic acid (IAA) is the most abundant form (Lavy and Estelle 2016). The distribution of auxin within plant tissues is controlled by biosynthesis, transport and inactivation, and once sensed by its receptors in the nucleus, triggers a series of downstream reactions (Zazimalova et al. 2010; Kasahara 2016). The core auxin sensing complex includes three parts: the F-box type auxin co-receptor TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX PROTEIN (TIR1/AFB), the transcriptional repressor Auxin/INDOLE-3-ACETIC ACID (Aux/IAA), and AUXIN RESPONSE FACTOR (ARF) (Salehin et al. 2015; Fig. 2C). Auxin enhances the interaction between the TIR1/AFB complex and Aux/IAA proteins, leading to degradation of Aux/IAA and release of ARF to regulate auxin-mediated transcriptional activation or repression of downstream genes (Okushima et al. 2005; Badescu and Napier 2006; Hagen 2015).

Inositol (pyro)phosphates not only play an important role in signaling P status, but are also involved in the auxin signaling pathway (Fig. 2C, Table1). TIR1 is an F-box protein containing a leucine-rich-repeat (LRR) that forms part of a SKP1/Cullin/F-box (SCF) type E3 ubiquitin ligase complex. Specifically, SKP1 (e.g., SKP1-like protein ASK1) links TIR1 to the Cullin (e.g., CUL1), which in turn interacts with RBX1, SCF-type ubiquitin ligases catalyze the transfer of activated

ubiquitin from a ubiquitin-conjugating enzyme (E2) to a target protein (i.e., Aux/IAAs) (Fig. 2C, Kepinski and Leyser 2005). The TIR1 protein expressed and purified from insect cells co-crystallized with $InsP_6$, while mutation of the InsPs/PP-InsPs binding site of TIR1 resulted in failure of the auxin-TIR1-Aux/IAA complex to form, suggesting that InsPs/PP-InsPs are directly involved in the auxin signaling pathway (Tan et al. 2007; Calderon Villalobos et al. 2012).

Addition of exogenous auxin induced the expression of the Arabidopsis *IPTK1* gene, and ITPK1 played an important role in auxin-mediated processes, including primary root elongation, leaf vein development, thermomorphogenesis and gravitropism (Laha et al. 2022). 5-$InsP_7$ produced by ITPK1 has a very high affinity for the auxin receptor TIR1, furthermore, 5-$InsP_7$ promotes the interaction between AFB1/AFB2 and Aux/IAA in yeast, suggesting that PP-InsPs are involve in auxin signaling (Laha et al. 2022).

Crosstalk between P status- and auxin- signaling

Root architecture undergoes adaptive changes, including the inhibition of primary root growth and the increase in the number and length of lateral roots under P-deficient conditions (Peret et al. 2011). It was reported that P deficiency changes the sensitivity of plant roots to auxin, which in turn causes the morphogenesis of plant lateral roots. Specifically, the expression of the auxin receptor gene *TIR1* is induced after P deficiency. As a result, the degradation of the repressor Aux/IAAs are accelerated, releasing ARF19, which further activates the expression of genes related to lateral root morphogenesis (Pérez-Torres et al. 2008). *AtPHR1*, a target gene of AtARF7 and AtARF19, is positively regulated by auxin signaling, and both AtPHR1 and its downstream PSI genes are down-regulated in *arf7*, *arf19*, and *arf7*/*arf19* mutants (Huang et al. 2018). In rice, knocking out *OsARF12* affected the transcript abundance of *OsPHR2* and its downstream genes, moreover, knocking out *OsARF16* resulted in the loss of primary root, lateral root and root hairs responses in response to auxin and P deficiency signals (Shen et al. 2013; Wang et al. 2014a), indicating that ARF family members are involved in the crosstalk between auxin signaling and P status. Although the *pho2* mutant (with high concentrations of Pi and $InsP_8$, Liu et al. 2012; Dong et al. 2019) and wild-type display similar phenotypes with respect to auxin responsiveness, the auxin insensitive primary root growth of *itpk1* plants is not observed anymore when plants are grown under P deficiency, indicating that both P overaccumulation and defective auxin responsiveness are independent consequences of impaired ITPK1 activity (Laha et al. 2022).

Given that $InsP_7$ and $InsP_8$ are sensitive to fluctuations in external P supply, whether P status affects the interaction between TIR1 and Aux/IAA at the protein level by controlling the synthesis of PP-InsPs, and then regulates plant growth and development is still elusive. It seems contradictory that P deficiency induces the expression of *TIR1*, but inhibits the synthesis of PP-InsPs, as they are both essential for the degradation of Aux/IAA and the release of ARFs. However, the underlying complex regulation mode is worthy of investigation (Fig. 2; Fig. 3).

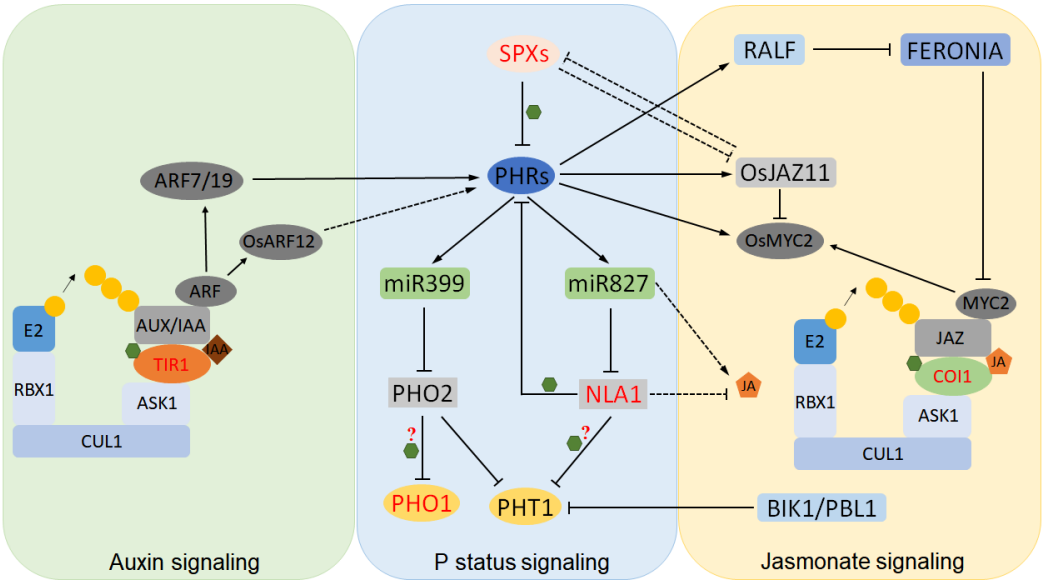

Fig. 3 The crosstalk among P status, auxin, and jasmonate signaling pathways in plants. Core transcription factor PHRs play major roles in the signaling crosstalk of P status, auxin, and JA. Firstly, PHRs regulate multi-pathways in P status signaling including microRNA-mediated surveillance of Pi uptake and transport. Secondly, *PHRs* targeted directly by ARF proteins so that auxin signaling is able to affect P status signaling. Thirdly, PHRs activate the expression of genes associated with JA signaling (e.g., *rapid alkalinization factor (RALF)*, *OsJAZ11*, and *OsMYC2*), in turn, protein kinases BIK1 and PBL1 in JA signaling regulate Pi uptake directly. Green hexagons indicate InsPs or PP-InsPs, and yellow circle indicates ubiquitin.

INOSITOL (PYRO)PHOSPHATES MEDIATED JASMONIC ACID SIGNALING PATHWAY

Inositol (pyro)phosphates and JA co-receptor COI1

Jasmonic acid (JA) is widely involved in plant growth and development, including root elongation, leaf senescence, and pollen fertility, and is also essential for plants to resist insect infestation, low temperature, drought and other stresses (Hu et al. 2017; Huang et al. 2017; Wang

et al. 2019a). JA is synthesized in chloroplasts and peroxisomes, and then chemically modified in the cytoplasm (Huang et al. 2017; Wang et al. 2019a). Methyl jasmonate (MeJA), JA-isoleucine complex (JA-Ile) and cis-jasmone (CJ) are biologically active JA derivatives, in which JA-Ile possesses the highest biological activity, and JASMONATE RESISTANT1 (JAR1) is responsible for its chemical modification (Wasternack and Strnad 2016; Wastenack and Song 2017).

When JA-lle is accumulated in plants, the COI1-JAZ protein complex acts as a JA co-receptor to bind to JA-lle, promoting the ubiquitination of the repressor JAZ proteins by the SCF-COI1 complex (Fig. 2D). After JAZ proteins are degraded by the 26S proteasome, the transcription factor MYCs are released and bind to the promoters of a series of JA-responsive genes, thereby turning on the expression of downstream genes (Fig. 2D). Similar to TIR1, COI1 is an F-box protein that forms part of a SKP1/Cullin/F-box (SCF) type E3 ubiquitin ligase complex (Chini et al. 2009; Kazan and Manners 2012; 2013). The COI1-JAZ co-receptor contains not only a JA-lle binding site, but also a InsPs or PP-InsPs binding site, indicating InsPs and PP-InsPs play important regulatory roles in the JA signaling pathway (Sheard et al. 2010; Laha et al. 2015; Fig. 2D, Table 1). Yeast two-hybrid experiments showed that the COI1 protein, mutated at the InsPs or PP-InsPs binding site, had a reduced interaction with the JAZ9 protein, and its mutant version also had a reduced degree of rescue to the inhibited phenotype of root growth and silique development in the *coi1* mutant, indicating that InsPs or PP-InsPs is indispensable for a functional COI1 (Mosblech et al. 2011). Yeast *ipk1Δ* strongly accumulates PP-$InsP_4$ (an inositol pyrophosphate), and the interaction between COI1 and JAZ9 is enhanced in yeast *ipk1Δ* mutant lines (Saiardi et al. 2002; Mosblech et al. 2011). Both the *ipk1* mutant and *vih2* mutants display a strong reduction of $InsP_8$, moreover, the phenotypes of *ipk1* mutant are similar to that of *vih2* plants that display compromised JA-dependent defenses (Laha et al. 2015; Laha et al. 2016). Based on bioinformatics analysis and radioligand reconstitution experiments, $InsP_8$ and COI1-JAZ co-receptor show a very high binding ability (Cui et al. 2018). The above findings indicate PP-InsPs, in particular $InsP_8$, is a co-ligand of the COI1-JAZ co-receptor and is essential for JA-mediated plant immune responses.

The content of PA in the mutants of genes related to PA synthesis pathway is decreased, and the immunity of the mutant lines to pathogenic bacteria is also decreased (Murphy et al. 2008). Potato inositol-3-phosphate synthase (MIPS) RNAi lines have reduced $InsP_6$ content and reduced

immunity to potato Y virus and tobacco mosaic virus (TMV), suggesting that $InsP_6$ maintains plant resistance to basic immunity to pathogens (Murphy et al. 2008). The function of multiple immune pathways in plants depends on the biosynthesis of InsPs and PP-InsPs. In *ipk1*, *itpk1* and *vih2* mutants, constitutive activation of immune signaling results in enhanced resistance to *Pseudomonas syringae*, indicating that Arabidopsis IPK1, ITPK1, and VIH2 inhibited SA-dependent immune responses (Gulabani et al. 2022). After JA treatment, the biosynthesis of $InsP_8$ is induced in plants, and *VIH2* regulates the plant's ability to sense JA and resist to herbivorous insects and disease fungi (Laha et al. 2015).

Crosstalk between P status- and JA- signaling

There exists a crosstalk between P status- and JA- signaling in plants, enabling plants to coordinately adapt to stresses such as P deficiency, pest invasion, and diseases (Fig. 3). In Arabidopsis, P deficiency signals can enhance JA synthesis and affect signaling pathways, thereby enhancing plant resistance to herbivorous insects (Khan et al. 2016). In cotton, JA synthesis is also increased under P deficiency, and the resistance of cotton to *Verticillium wilt* is greatly enhanced (Luo et al. 2021). The *GhAOS* gene RNAi lines have a weakened resistance to *Verticillium wilt* under P deficiency, indicating that P deficiency signals enhanced cotton's resistance to *Verticillium wilt* by activating JA biosynthesis (Luo et al. 2021). Transcriptome analysis revealed that the differential expression of JA- and SA-related genes during P deficiency is dependent on PHR1, suggesting that PHR1 can regulate plant immune responses at the transcriptional level (Castrillo et al. 2017). Recently, it was reported that AtPHR1 activates the expression of *rapid alkalinization factor (RALF)* under P-deficient conditions, subsequently, RALF inhibits the complex formation of pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) receptor through the PTI modulator FERONIA (Tang et al. 2022). Impairment of the plant immune response via the PHR1-RALF-FERONIA pathway allows the colonization of root-specific microbial communities, which in turn alleviate the PSR (Tang et al. 2022). The resistance of rice to *Xanthomonas oryzae* pv. *oryzae* is enhanced under P deficiency, which is achieved via OsPHR2 by activating the expression of the core transcription factor MYC2 in the JA signaling pathway (Kong et al. 2021). After benzoic acid treatment or inoculation with *Pseudomonas syringae* pv *tomato* DC3000, SA accumulated in *nla1 (bah1)* mutants, suggesting that *NLA1/BAH1* (*benzoic acid hypersensitive1*) is involved in plant immune responses by regulating benzoic acid- and pathogen-induced SA

accumulation (Yaeno and Iba 2008). The P concentration in the *nla1* mutant and *miR827*-overexpressing lines was elevated, resulting in increased resistance to *Plectosphaerella cucumerina* (Val-Torregrosa et al. 2022). When infected with pathogenic bacteria or treated with fungal inducers, the expression of *miR827* is induced, while the expression of *NLA1* is down-regulated. Moreover, the concentrations of callose, phytoalexin, SA and JA in the leaves of *nla1* mutants are increased, indicating that NLA1 may be a negative regulator involved in plant immunity (Val-Torregrosa et al. 2022). NLA1 may control the JA signaling pathway by regulating the protein level of PHR1 with a PP-InsPs-dependent manner (Park et al. 2022; Fig. 3).

P deficiency signals can affect JA biosynthesis and signaling pathways, and the key genes of JA biosynthesis and signaling pathways are also involved in the PSR of plants (Khan et al. 2016; Pandey et al. 2021; Fig. 3). The P deficiency inducible gene *OsJAZ11* is regulated by OsPHR1 at the transcriptional level, and overexpression of *OsJAZ11* alleviates the inhibitory effect of JA on rice root growth (Pandey et al. 2021). *OsJAZ11* overexpression lines have an increased primary and seminal root elongation, and their ability to forage P is enhanced (Pandey et al. 2021). The PSI genes are significantly down-regulated in *OsJAZ11*-overexpressing lines, whereas they are significantly up-regulated in RNAi lines, indicating that OsJAZ11 suppressed the PSR (Pandey et al. 2021). OsJAZ11 protein can interact with OsSPX1 protein, which may be another way of regulating PSR (Pandey et al. 2021). Recently, it was reported that protein kinases BIK1 and PBL1 functioning in immune pathway inhibit the activity of PHT1;4 via phosphorylation, suggesting that activation of immune signaling can directly inhibit Pi uptake in plants (Dindas et al. 2022).

CONCLUSION AND FUTURE PERSPECTIVE

In the past two decades, great progress has been achieved in the biosynthetic pathways of InsPs and PP-InsPs and their emerging roles in P status, auxin and JA signaling pathways in plants. The regulation of PP-InsPs on the SPX domain protein subfamily has been clearly elucidated. However, the dependence of the SPX-EXS, SPX-MFS and SPX-RING subfamily members on PP-InsPs still needs further study to understand their molecular mechanisms of controlling plant P status. In addition, given that PP-InsPs are essential for signaling P status, auxin and JA signaling pathways, whether P status acts on phytohormone signaling pathway by controlling the synthesis

of PP-InsPs, and in turn phytohormone signaling affects the growth and development of plants under different P supply also warrants further study. Although it is becoming clearer that the SPX domain containing proteins are intracellular sensors, it is largely unknown what kind of proteins act as local P sensors. Understanding of whether and how PP-InsPs integrate local and systemic signaling pathways to module plant P status will be beneficial for genetic improvement of crop P efficiency.

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Author contributions

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