Tansley review

The good, the bad, and the phosphate: regulation of beneficial and detrimental plant–microbe interactions by the plant phosphate status

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Summary

Phosphate (P_i) is indispensable for life on this planet. However, for sessile land plants it is poorly accessible. Therefore, plants have developed a variety of strategies for enhanced acquisition and recycling of P_i. The mechanisms to cope with P_i limitation as well as direct uptake of P_i from the substrate via the root epidermis are regulated by a conserved P_i starvation response (PSR) system based on a family of key transcription factors (TFs) and their inhibitors. Furthermore, plants obtain P_i indirectly through symbiosis with mycorrhiza fungi, which employ their extensive hyphal network to drastically increase the soil volume that can be explored by plants for P_i. Besides mycorrhizal symbiosis, there is also a variety of other interactions with epiphytic, endophytic, and rhizospheric microbes that can indirectly or directly influence plant P_i uptake. It was recently discovered that the PSR pathway is involved in the regulation of genes that promote formation and maintenance of AM symbiosis. Furthermore, the PSR system influences plant immunity and can also be a target of microbial manipulation. It is known for decades that the nutritional status of plants influences the outcome of plant–microbe interactions. The first molecular explanations for these observations are now emerging.

I. Introduction

Phosphorous (P) is one of the most critical macronutrients in living organisms. It is an indispensable constituent of a plethora of biological molecules, including nucleic acids, phospholipids, proteins, and metabolites. Additionally, P in the form of orthophosphate (P_i) is needed for phosphorylation,

an important and frequent event with regulatory and signaling functions in living cells. A major problem concerning the acquisition of P_i lies in its limited mobility and scarce availability in soils and mineral environments (Schachtman *et al.*, 1998). Thus, primary producers such as microorganisms and plants needed to evolve strategies to acquire P_i from their surroundings.

Sufficient P_i supply to agricultural systems is crucial to maintain the productivity of crop plants as the major cornerstone of human nutrition. Thus, the availability of rock P_i as base for P_i fertilizer is of major economic and geopolitical importance (Reijnders, 2014). However, the extensive use of P_i fertilizer in agriculture leads to enrichment of heavy metals like uranium in agricultural ecosystems and the food chain (Schnug & Haneklaus, 2015) and the eutrophication of groundwater by washout of superfluous P_i can lead to harmful algal blooms (Ferro *et al.*, 2015). To avoid P_i loss from agricultural fields, it would be desirable to efficiently synchronize P_i input with uptake by crops.

To acquire P_i, plants evolved an arsenal of high- and low-affinity transport proteins, phosphatases, and secreted organic acids, which allows solubilization of mineral P_i and its uptake from the rhizosphere (Lambers *et al.*, 2006). Plants take up P_i via the direct uptake pathway through the rhizodermis (Bucher, 2007; Y. Wang *et al.*, 2021). However, due to the very limited mobility of P_i in soils, a P_i depletion zone develops rapidly around roots (Hinsinger *et al.*, 2005). To reach for P_i deposits beyond the P_i depletion zone, the plant needs to form new lateral roots, which is a cost-intensive developmental process (Péret *et al.*, 2014; Gutiérrez-Alanís *et al.*, 2018; Huang & Zhang, 2020). The risk arises for the plant, if investing in a larger root system provides sufficient nutrients to be profitable.

To overcome this problem, *c.* 80% of land plants engage in the mutualistic arbuscular mycorrhiza (AM) symbiosis with fungi from the phylum Glomeromycotina. The fungi supply plants with water and mineral nutrients and in turn receive essential carbon sources in the form of hexoses and lipids (Wang *et al.*, 2017; Wipf *et al.*, 2019). AM fungi take up mineral nutrients from the soil via extensive hyphal networks and transport them to the root (Chen *et al.*, 2018). In the case of P_i, this may be promoted by a bacterial community colonizing the hyphosphere that promotes organic P_i mineralization (Wang *et al.*, 2022). The nutrients are then released to the plant via tree-shaped hyphal structures, called arbuscules that form inside root cortex cells (Luginbuehl & Oldroyd, 2017). The mycorrhizal uptake route for nutrients is also referred to as the indirect Pi uptake pathway (Smith & Smith, 2011).

Woody plants from temperate and cold regions engage into ectomycorrhizal symbioses with Ascomycetes and Basidiomycetes that support them with nutrients derived from minerals and decomposed organic matter (Landeweert *et al.*, 2001; Lindahl & Tunlid, 2015). In contrast to AM symbiosis, ectomycorrhizal fungi deliver nutrients to the inside of roots by extracellular hyphal structures (Martin *et al.*, 2016).

Plants have evolved a P_i starvation response (PSR) system that dynamically regulates the direct P_i uptake pathway to meet the nutritional requirements of the plant in changing environments. This system is already present in green algae and streptophytes (Rubio *et al.*, 2001; Rico-Reséndiz *et al.*, 2020) and was retained across the land plant phylogeny.

The PSR system interacts with further pathways regulating nutrition, growth, development, and environmental interactions. It was demonstrated to be directly involved in the regulation of the coordinated uptake and distribution of other mineral nutrients such as nitrate, iron, and sulfur together with P_i (Rouached

et al., 2011; Bournier *et al.*, 2013; Hu *et al.*, 2019; X. Wang *et al.*, 2020). Furthermore, it acts as an integrator of various plant physiological stimuli and conditions, such as light and phytohormone signaling (Liu *et al.*, 2017; Huang *et al.*, 2018) or the availability of nitrogen, zinc, and sugar (Karthikeyan *et al.*, 2007; Khan *et al.*, 2014; Medici *et al.*, 2019). Besides P_i deficiency, the PSR system is also crucial for the alleviation of other abiotic stresses like high light conditions and hypoxia (Kistner & Parniske, 2002; Nilsson *et al.*, 2012).

While for a long time the PSR system was mainly implicated in plant interaction with the abiotic environment, it was in recent years also linked to plant interactions with microbes. It was shown to regulate the ability of plants, to form AM symbiosis (P. Wang *et al.*, 2021; Shi *et al.*, 2021; Das *et al.*, 2022; Liao *et al.*, 2022), which closed a major knowledge gap on how AM symbiosis is promoted at low and suppressed at high P_i conditions (Breuillin *et al.*, 2010; Balzergue *et al.*, 2011). Besides AM symbiosis, also the root nodule endosymbiosis between legumes and nitrogen-fixing rhizobia bacteria is highly responsive to the plant P_i status and under control of the PSR system (Ma & Chen, 2021). The association with endophytic fungi was shown not only to be regulated but also to regulate the PSR system (Hiruma *et al.*, 2016; Bakshi *et al.*, 2017). In addition, immune responses to detrimental microbes and the modulation of bacterial community compositions in the rhizosphere via the plant immune system are governed by the plant P_i status and the PSR system (Castrillo *et al.*, 2017; Finkel *et al.*, 2019; Dindas *et al.*, 2022; Tang *et al.*, 2022). The influence of the plant P_i status on plant–microbe interactions has been observed for a long time, and the molecular underpinnings of these phenomena are now being revealed.

II. The P_i starvation response system in plants

1. The role of PHR proteins and their regulatory targets

Central players of the P_i starvation response (PSR) system are transcriptional regulators named Phosphate Starvation Response (PHR; Rubio *et al.*, 2001). These are conserved from unicellular green algae to vascular plants (Rubio *et al.*, 2001; Rico-Reséndiz *et al.*, 2020) and belong to the class of Myeloblastoma-coiled-coil TFs (Myb-CC TFs).

Their coiled-coil domain is involved in the formation of homodimers (Rubio *et al.*, 2001; Guan *et al.*, 2022) that bind to the Phosphate Starvation Response 1 binding site (*P1BS*), a partially palindromic sequence motif (GNATATNC) frequently found in promoter regions of genes involved in PSR (Fig. 1; Franco-Zorrilla *et al.*, 2004; Schünmann *et al.*, 2004a,b; Bustos *et al.*, 2010). Some of the most prominent regulatory targets of PHR1 are genes encoding high-affinity P_i transporters of the Phosphate Transporter 1 (PHT1) family (Nilsson *et al.*, 2007; Z. Wang *et al.*, 2014; for review on different types of P_i transporters and their molecular characteristics, see Rausch & Bucher, 2002; F. Smith *et al.*, 2003; Raghothama, 2005; Wang *et al.*, 2018), purple acid phosphatases (PAPs; Nilsson *et al.*, 2007; Zhang *et al.*, 2011), SPX domain (named after the proteins in which they were discovered: yeast SYG1, PHO81 and human Xpr1) containing proteins (Puga
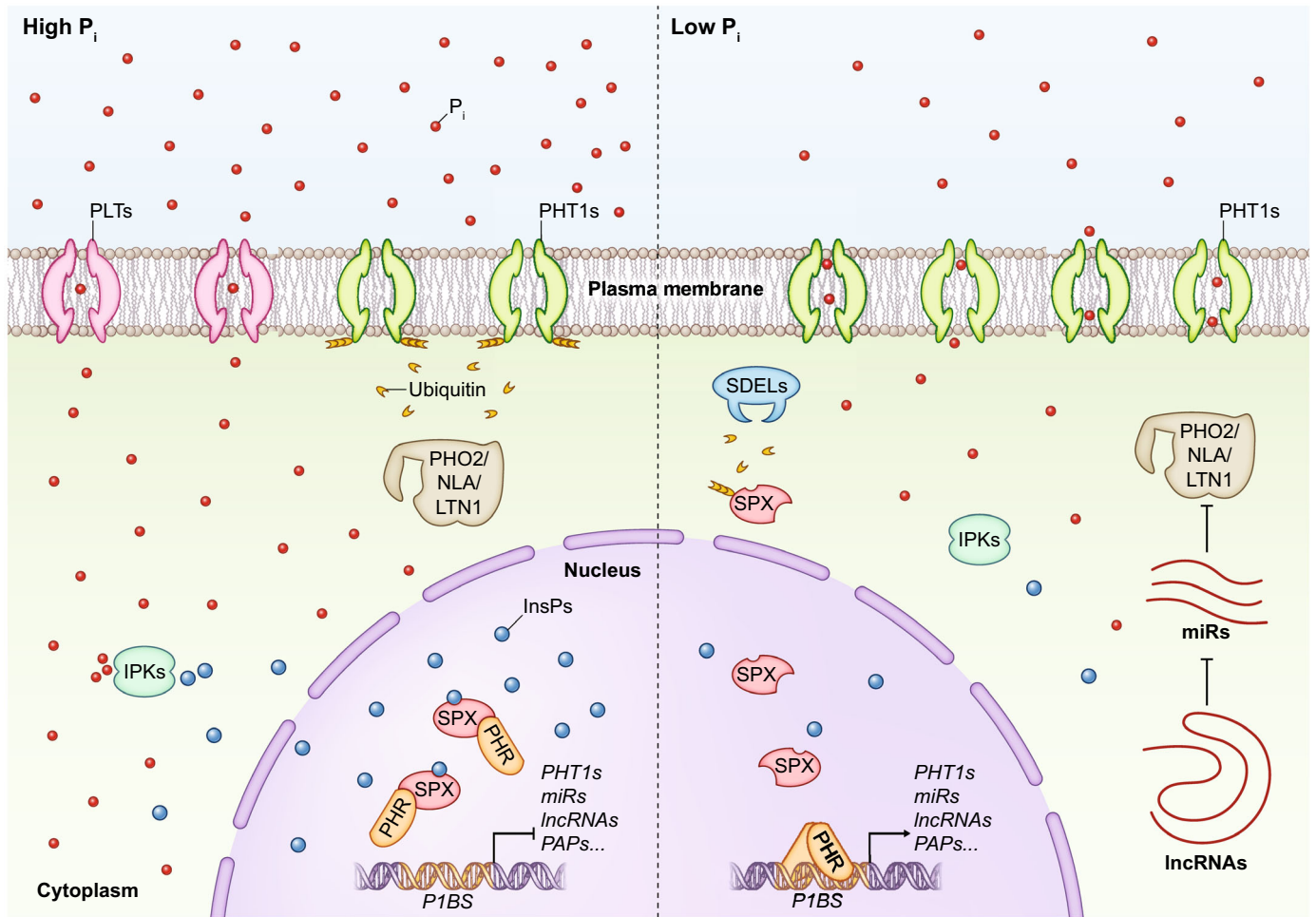
Fig. 1 Core components of the phosphate starvation response (PSR) system. Under high P_i conditions, low-affinity P_i transporters (PLTs) import cellular P_i, which is incorporated into inositol phosphate (InsP) molecules (Bennett *et al.*, 2006; Lambers *et al.*, 2006; Wilson *et al.*, 2013). These are sensed by SPX domain (named after the proteins in which they were discovered: yeast SYG1, PHO81 and human Xpr1) proteins that inhibit the action of PHOSPHATE STARVATION RESPONSE (PHR) transcription factors (Lv *et al.*, 2014; Puga *et al.*, 2014; Z. Wang *et al.*, 2014; Zhong *et al.*, 2018). Ubiquitin ligating enzymes like PHOSPHATE 2 (PHO2), LEAF TIP NECROSIS 1 (LTN1), and NITROGEN LIMITATION ADAPTATION (NLA) mark high-affinity P_i transporters (PHTs) for proteasomal degradation by ubiquitination (Aung *et al.*, 2006; Bari *et al.*, 2006; Chiou *et al.*, 2006; Hu *et al.*, 2011; Lin *et al.*, 2013a). At P_i starvation, SPX proteins are not bound to InsP, are inactive, and are marked for proteasomal degradation via the SDEL RING-Finger ubiquitin ligases (Ruan *et al.*, 2019). PHR transcription factors form homodimers and bind their target motif *P1BS* in the DNA to activate transcription of PSR genes (Rubio *et al.*, 2001; Schünmann *et al.*, 2004a,b; Franco-Zorrilla *et al.*, 2007; Bustos *et al.*, 2010; Guan *et al.*, 2022). Besides PHT-encoding genes, these include genes coding for RNA molecules involved post-transcriptional silencing of ubiquitin-conjugating enzyme transcripts (Fujii *et al.*, 2005; Bari *et al.*, 2006; Franco-Zorrilla *et al.*, 2007; Nilsson *et al.*, 2007; Bustos *et al.*, 2010; Gu *et al.*, 2010; Lundmark *et al.*, 2010; Z. Wang *et al.*, 2014; Yuan *et al.*, 2016).

et al., 2014; Z. Wang *et al.*, 2014), the miRNA genes of the miR399 family, the expression levels of which are strongly increased upon P_i starvation (Fujii *et al.*, 2005; Bari *et al.*, 2006), and the long noncoding RNA (lncRNA) *Induced by Phosphate Starvation 1* (*IPS1*), which sequesters miR399 members by target mimicry (Fig. 1; Franco-Zorrilla *et al.*, 2007; Bustos *et al.*, 2010; Yuan *et al.*, 2016). Also other miRNAs, such as miR827, were reported to be induced at low P_i status as important intercellular signals of PSR (Gu *et al.*, 2010; Lundmark *et al.*, 2010). miRNAs of the miR399 and miR827 family target the transcripts of negative regulators of PSR, like the ubiquitin-conjugating enzymes PHOSPHATE 2 (PHO2), LEAF TIP NECROSIS 1 (LTN1), and NITROGEN LIMITATION ADAPTATION (NLA), for post-transcriptional silencing (Fig. 1; Aung *et al.*, 2006; Bari *et al.*, 2006; Chiou *et al.*, 2006; Hu *et al.*, 2011; Lin *et al.*, 2013a).

These ubiquitin-conjugating enzymes are major executors of proteasomal degradation of high-affinity P_i transporters (Hu *et al.*, 2011; Liu *et al.*, 2012; Park *et al.*, 2014; Yue *et al.*, 2017), as well as of factors involved in P_i transporter shuttling from the endoplasmic reticulum, like the SEC12-related protein PHOSPHATE TRANSPORTER TRAFFIC FACILITATOR 1 (PHF1; González *et al.*, 2005). Thereby, these ubiquitin-conjugating enzymes attenuate the PSR by regulating protein abundance and localization of high-affinity P_i transporters.

Phosphate Starvation Response proteins not only induce genes encoding components of the direct P_i uptake machinery and its regulators but also of indirect mechanisms of P_i recycling, such as dephosphorylation of phospholipids (Acevedo-Hernández *et al.*, 2012; Pant *et al.*, 2015a) and the biosynthesis of anthocyanins (Nilsson *et al.*, 2007). Additionally, PHRs are involved in

remodeling the chromatin landscape and play a crucial role in changing chromatin accessibility and transcription of the associated (PSR) genes in response to P_i starvation (Barragán-Rosillo *et al.*, 2021). They are also responsible for major metabolic changes in response to P_i starvation (Morcuende *et al.*, 2007; Pant *et al.*, 2015a,b), like the accumulation of proline for enhanced stress adaptation (Aleksza *et al.*, 2017).

In vitro and *in vivo* experiments showed sumoylation activity of SAP AND MIZ1 DOMAIN-CONTAINING LIGASE 1 (SIZ1) with PHR1 as a likely target (Miura *et al.*, 2005). However, the role of PHR1 sumoylation is not entirely clear: transcript accumulation of some PSR genes is reduced in *siz* mutants, pointing toward an activation of PHR1 via sumoylation while the transcript levels of other PSR genes are increased, suggesting the opposite (Miura *et al.*, 2005). Furthermore, *siz* mutants are hypersensitive to P_i starvation, which rather indicates that SIZ1 suppresses PSR and is possibly involved in its fine-tuning.

Consistent with the important molecular function of PHRs, phosphate starvation responses in *phr* mutants are strongly perturbed. For example, *phr* mutants of *Arabidopsis* show reduced anthocyanin content, root-to-shoot ratio, root hair length, and lower induction of PSR genes under phosphate starvation (Rubio *et al.*, 2001; Bustos *et al.*, 2010). Furthermore, the uptake of P_i is reduced even under P_i-sufficient conditions, which likely explains the reduced fresh weight of the mutant (Rubio *et al.*, 2001). Consistently, the overexpression of PHR1 causes an increase in P_i uptake in *Arabidopsis* and rice (Nilsson *et al.*, 2007; Z. Wang *et al.*, 2014).

2. The role of SPX domain proteins

The abundance of P_i within cells is monitored by proteins that contain SPX domains (named after the proteins in which they were discovered: yeast SYG1, PHO81, and human Xpr1). SPX domains sense the cellular P_i status by concentration-dependent binding to inositol pyrophosphates (InsPs; Wild *et al.*, 2016), which are produced by inositol polyphosphate kinases (IPKs) and accumulate at P_i sufficiency (Fig. 1; Bennett *et al.*, 2006; Wilson *et al.*, 2013). SPX proteins directly interact with PHR proteins (and other PSR regulating plant TFs) in a P_i (inositol pyrophosphate)-dependent manner (Lv *et al.*, 2014; Puga *et al.*, 2014; Z. Wang *et al.*, 2014; Zhong *et al.*, 2018; Dong *et al.*, 2019; Chen *et al.*, 2021; He *et al.*, 2021). This impairs the nuclear migration and/or DNA binding of PHR proteins at high plant P_i status (Fig. 1; Lv *et al.*, 2014; Puga *et al.*, 2014; Z. Wang *et al.*, 2014; Zhong *et al.*, 2018). At P_i starvation, SPX proteins are marked for proteasomal degradation by SPX4 Degradation E3 Ligase (SDEL) RING-finger ubiquitin ligases (Fig. 1; Ruan *et al.*, 2019). The P_i-dependent interaction with SPX proteins occurs through the coiled-coil domains in PHRs that are also important for homodimerization (Rubio *et al.*, 2001; Ried *et al.*, 2021; Guan *et al.*, 2022).

III. The role of the PSR system in AM symbiosis

Arbuscular mycorrhiza symbiosis provides an additional, and possibly the predominant, route for P_i uptake for a majority of plants in nature (S. Smith *et al.*, 2003). Germination and growth of AM fungi are induced by strigolactones (SLs) that are exuded from plant roots at low P_i status (Fig. 2a; Akiyama *et al.*, 2005; Besserer *et al.*, 2006, 2008; Yoneyama *et al.*, 2007a,b; López-Ráez *et al.*, 2008). Also flavonoids that promote root colonization by AM fungi (Nair *et al.*, 1991; Akiyama *et al.*, 2002) are enriched in the root exudates of P_i-starved plants (Akiyama *et al.*, 2002; Malusà *et al.*, 2006). In turn, AM fungi release (lipo-)chitooligosaccharides (Maillet *et al.*, 2011; Genre *et al.*, 2013), which are perceived by LysM receptor-like kinase complexes containing CHITIN

Fig. 2 Regulation of arbuscular mycorrhiza (AM) symbiosis by the plant phosphate starvation response (PSR) system, as described in *Oryza sativa*, *Lycopersicon esculentum*, and *Medicago truncatula*. (a) At P_i starvation, the PSR system promotes the expression of genes involved in the formation and functioning of AM symbiosis (P. Wang *et al.*, 2021; Shi *et al.*, 2021; Das *et al.*, 2022; Liao *et al.*, 2022). Low cellular amounts of inositol phosphates (InsPs) lead to degradation of SPX (named after the proteins in which they were discovered: yeast SYG1, PHO81 and human Xpr1) proteins, releasing PHOSPHATE STARVATION RESPONSE (PHR) transcription factors from sequestration and cytoplasmic retainment (Lv *et al.*, 2014; Puga *et al.*, 2014; Z. Wang *et al.*, 2014; Zhong *et al.*, 2018). This allows their homodimerization, nuclear migration, and DNA binding to *P1BS* sequence motifs (Lv *et al.*, 2014; Puga *et al.*, 2014; Z. Wang *et al.*, 2014; Zhong *et al.*, 2018; Ried *et al.*, 2021; Guan *et al.*, 2022). Thereby, they activate transcription of strigolactone (SL) biosynthesis genes (*CAROTENOID CLEAVAGE DIOXYGENASE 8*, *CCD8*) and genes encoding their transcriptional regulators (*NODULATION SIGNALING PATHWAY2*, *NSP2*; Das *et al.*, 2022; Liu *et al.*, 2011). The increased exudation of SLs promotes spore germination, hyphal branching, and Myc factor exudation by Glomeromycotina fungi. The PSR system also promotes transcription of epidermal surface receptor encoding genes (*CHITIN ELICITOR RECEPTOR KINASE1*, *CERK1*, and *SYMBIOSIS RECEPTOR KINASE*, *SYMRK*) that form complexes involved in the perception of fungal signaling molecules enabling fungal entry; and the transcription of a zaxinone synthase (*ZAS*) gene, encoding an enzyme that synthesizes the apocarotenoid compound zaxinone (Das *et al.*, 2022) that promotes root colonization by AM fungi (Wang *et al.*, 2019; Votta *et al.*, 2022). In the inner layers of the root cortex, PHR transcription factors activate transcription factor encoding genes (*REQUIRED FOR ARBUSCULAR MYCORRHIZATION1*, *RAM1*, and *RAD1*) that enable the fine branching of arbuscules and the expression of genes encoding peri-arbuscular membrane (PAM)-localized phosphate and ammonium transporters (PTs and AMTs), which are also direct target of PHRs (Shi *et al.*, 2021; Das *et al.*, 2022). (b) In *Medicago*, it was described that SPX1 and 3 may be involved in the regulation of arbuscule lifetime, because the *spx1,3* double mutant contains a higher proportion of mature vs degraded arbuscules (P. Wang *et al.*, 2021). It is unknown how this occurs, but the SPX proteins could be involved in regulating arbuscule turnover when P_i levels in the arbuscule-containing cells become too high after arbuscule formation, PHOSPHATE TRANSPORTER 4 (PT4) expression and P_i import. This would be consistent with a model in which PHR or another protein inhibits the expression of genes associated with arbuscule degradation, such as *CYSTEINE PROTEASE3* (*CP3*) and a chitinase either by direct binding to their promoter or by sequestering their positive regulator. Indeed, these genes are poorly expressed in the *spx1,3* double mutant. Hypothetically, upon local P_i increase in arbuscule-containing cells, the co-induced and activated SPX1 and 3 may sequester PHRs and/or the additional inhibitor, thereby reducing *PT* expression and derepression of genes associated with arbuscule collapse, finally leading to the initiation of the arbuscule degradation program. This way, an increased abundance and activity of SPX proteins in arbuscule-containing cells causes a shift from high levels of colonization and a high ratio of mature to degenerating arbuscules, to low overall colonization and a reduced ratio of mature to degenerating arbuscules (P. Wang *et al.*, 2021).

(a) *Oryza sativa*   *Solanum lycopersicum*   **(b)** *Medicago truncatula*
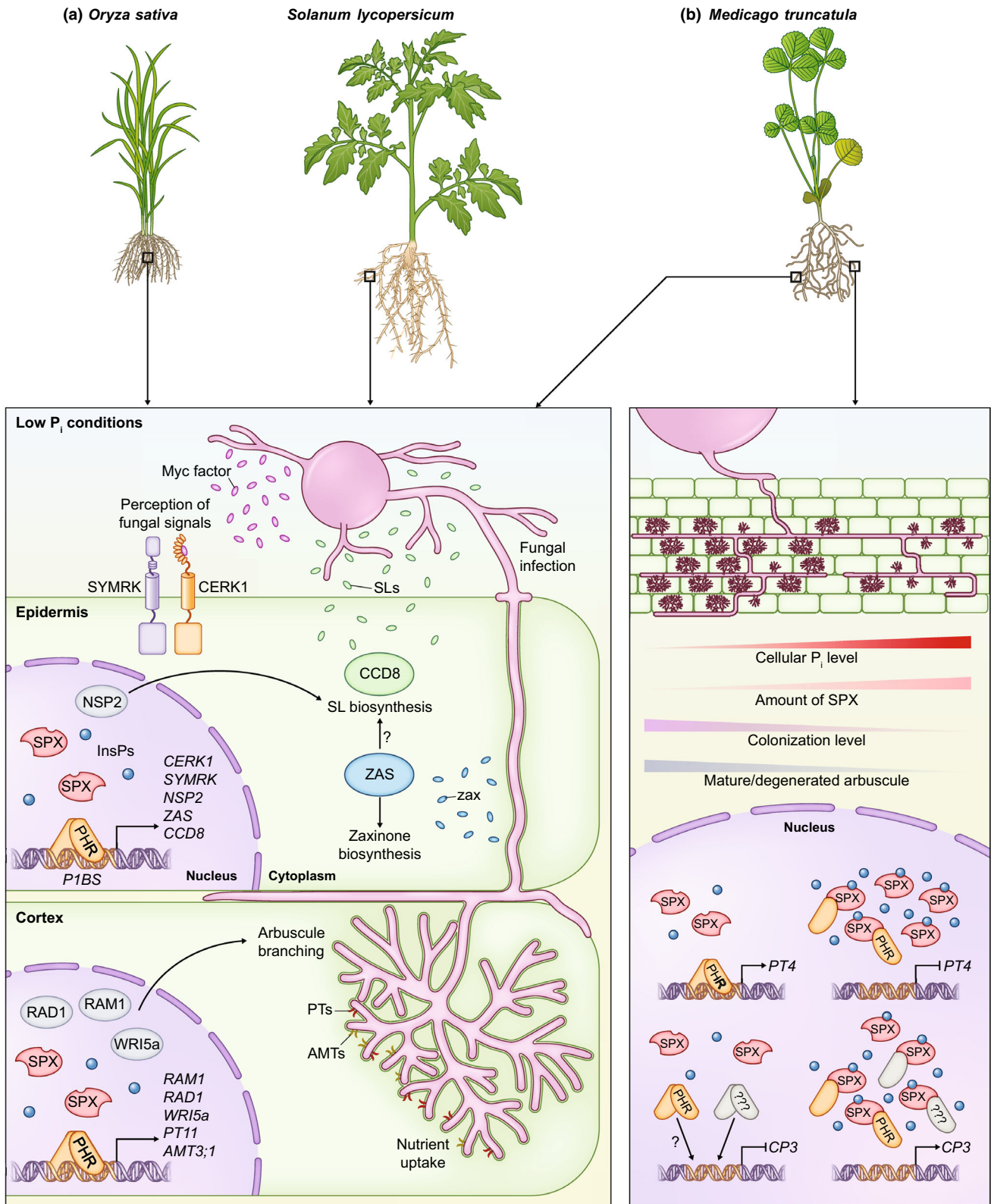
ELICITOR RECEPTOR KINASE 1 (CERK1) and Nod Factor Receptor 5/Nod Factor Perception (NFR5/NFP) orthologs in rice and *Medicago truncatula* (Fig. 2a; Feng *et al.*, 2019; He *et al.*, 2019).

Once the fungus makes contact with the root surface, it forms an attachment structure, the hyphopodium, and enters the root through or between epidermal cells (Fig. 2a). Root entry requires a

so-called common symbiosis signaling network, which is also needed for nitrogen-fixing root nodule symbiosis (Oldroyd, 2013). Once the fungus reaches the inner cortex, it inserts hyphal protrusions into cells that develop into highly branched arbuscules, surrounded by a plant-derived peri-arbuscular membrane (PAM), at which nutrients are exchanged between the symbionts (Fig. 2a; Luginbuehl & Oldroyd, 2017). The exact mechanisms underlying this exchange are still not fully understood, though it is known that paralogs of canonical plant nutrient transporter genes are involved, the products of which are guided to the PAM through specific coordination of their temporal expression with a reorientation of secretion during arbuscule development (Javot *et al.*, 2007; Guether *et al.*, 2009; Pumplin *et al.*, 2012; An *et al.*, 2019). Similar to P_i transporters operating in direct uptake, mycorrhizal P_i uptake also appears to rely on proton gradients over cellular membranes generated by a PAM-localized ATPase required for phosphate transfer and arbuscule branching (Krajinski *et al.*, 2014; E. Wang *et al.*, 2014). This makes it likely that the mycorrhizal P_i importers in the PAM act as H^+/P_i symporters (F. Smith *et al.*, 2003).

1. The PSR system directly regulates AM development and functioning

It has been known for a long time that the plant P_i status influences the formation of AM (Graham *et al.*, 1981; Thomson *et al.*, 1986; Elias & Safir, 1987): P_i depletion leads to a promotion, P_i sufficiency to a repression of root colonization. Split root experiments with pea and petunia impressively revealed that AM is regulated systemically by the phosphate status, since it was sufficient to supply high P_i fertilizer to one side of the split root to suppress AM on the other side although it was fertilized with low P_i (Breuillin *et al.*, 2010; Balzergue *et al.*, 2011). Systemic repression of AM upon high P_i may involve CLAVATA3/Embryo Surrounding Region-Related (CLE)-peptides such as CLE33. *CLE33* is induced after high P_i treatment in *M. truncatula* roots and causes a reduction of AM in a *SUPER NUMERIC NODULES* (*SUNN*)-dependent manner when it is ectopically expressed in roots under the control of the *35S* promoter (Müller *et al.*, 2019). SUNN is a leucine-rich repeat receptor-like kinase (LRR-RLK) that negatively controls nodulation as well as colonization by AM fungi, systemically from the shoot (Meixner *et al.*, 2005; Schnabel *et al.*, 2005). Systemic promotion of AM under low P_i may involve members of the miR399 family as they are important players in systemic communication of the plant P_i status (Fujii *et al.*, 2005; Bari *et al.*, 2006; Chiou *et al.*, 2006). Indeed, *miR399* expression levels change between different P_i conditions and during AM symbiosis (Branscheid *et al.*, 2010; Gu *et al.*, 2010; Xu *et al.*, 2018). However, genetic evidence for an involvement of miR399 in regulating AM is still lacking.

A number of AM-induced genes specifically expressed in arbuscule-containing cells harbor *P1BS* elements within their regulatory sequences (Lota *et al.*, 2013). Therefore, it was tempting to speculate that the P_i-dependent AM formation is regulated by the PHR-SPX system (Carbonnel & Gutjahr, 2014). Indeed recently, the direct link between PSR signaling and AM symbiosis

development was uncovered (P. Wang *et al.*, 2021; Shi *et al.*, 2021; Das *et al.*, 2022). In rice, a Y1H screen with 51 preselected promoters against 1570 rice TFs identified the PHR family member OsPHR2 as central hub in a transcriptional network of genes expressed in arbuscule-containing cells (Fig. 2a; Shi *et al.*, 2021). Furthermore, a combination of transcriptomics with ChIP-Seq revealed that OsPHR2 is a major player in the priming of roots for AM symbiosis (Fig. 2a; Das *et al.*, 2022). At low P_i conditions already in absence of the fungus, a number of genes involved in AM symbiosis are directly targeted by PHR2, contain the *P1BS* element in their promoter, and are less expressed in *phr2* mutants as compared to the wild-type (WT; Das *et al.*, 2022). Among them are SL biosynthesis genes and LysM-RLKs involved in precontact signaling, common symbiosis genes required for fungal cell entry such as *SYMBIOSIS RECEPTOR KINASE* (*SYMRK*), *CALCIUM and CALMODULIN DEPENDENT KINASE (CCaMK)* and *CYCLOPS* and genes encoding nutrient transporters, which operate in nutrient exchange at the peri-arbuscular membrane (Fig. 2a; Das *et al.*, 2022; Shi *et al.*, 2022). For the common symbiosis genes SYMRK, CCaMK, and CYCLOPS, this was confirmed in the *phr1a* mutant of the model legume and dicotyledon *Lotus japonicus* (Das *et al.*, 2022). In addition, Shi *et al.* (2021), Das *et al.* (2022), and Liao *et al.* (2022) showed direct binding of PHRs to *P1BS* elements in the promoter regions of key genes operating in AM symbiosis. Interestingly, *CCaMK* and *CYCLOPS* are already present in genomes of Charophytes, suggesting a predisposition for symbiosis in the algal ancestors of land plants (Delaux *et al.*, 2015). It will be interesting to understand whether these genes were already wired to the PSR system in Charophyte algae, (which would suggest that they were already involved in phosphate starvation responses) and in which plant or algal clade this regulatory link evolved.

Consistent with an important function of PHRs in regulating symbiosis, mutation of OsPHR2 resulted in an almost complete loss of AM symbiosis (Shi *et al.*, 2021; Das *et al.*, 2022), while mutation in *L. japonicus* and virus-induced silencing in tomato of a single *PHR* family member led to a reduction in colonization (Das *et al.*, 2022; Liao *et al.*, 2022). Exogenous application of the synthetic SL analog *rac*-GR24 partially rescued colonization of rice *phr2* mutants (Das *et al.*, 2022), showing that the reduced expression of SL biosynthesis genes causing reduced SL exudation explains part of the phenotype. However, interestingly it could not rescue the low colonization of P_i-replete pea and petunia plants (Breuillin *et al.*, 2010; Balzergue *et al.*, 2011). This confirms that reduced SL exudation at high P_i is not the only cause for the suppression of AM at this condition and also suggests differences among plant species with respect to the importance of SL exudation for colonization.

Overexpression of *PHR2* partially restored root colonization in rice and in *L. japonicus* at high P_i conditions at which AM is suppressed in WT roots (Shi *et al.*, 2021; Das *et al.*, 2022), suggesting that SPX proteins are unable to titrate PHRs, when they occur at increased amounts. Indeed, SPX proteins play a negative role in AM of rice and tomato (Shi *et al.*, 2021; Liao *et al.*, 2022). Rice *spx1/2/3/5* quadruple mutants and tomato *spx1* mutants showed increased root colonization, while *SPX* overexpression in

both plant species led to reduced colonization (Shi *et al.*, 2021; Liao *et al.*, 2022).

Intriguingly, in the model legume *Medicago truncatula SPX1* and *SPX3* seem to play a partially different and unexpected role (Fig. 2b; P. Wang *et al.*, 2021). Both, ectopic expression of *SPX1* or *SPX3* under the control of the strong arbuscule-containing cell-specific *PHOSPHATE TRANSPORTER 4* (*PT4*) promoter as well as mutation of the two genes reduced AM colonization (P. Wang *et al.*, 2021). Based on seed germination assays of the parasitic weed *Phelipanche ramosa*, the authors concluded that the *mtspx1* and *3* mutants release reduced amounts of SL, which may explain the low colonization levels, but appears counterintuitive, as rice *phr2* also displayed reduced SL exudation (Das *et al.*, 2022). Simultaneously, the *spx1,3* double mutant displayed a higher ratio of mature/collapsed arbuscules, while expression of SPX1 and 3 under the control of the *PT4* promoter caused the opposite phenotype (Fig. 2b). This indicates that SPX1 and 3 may be involved in regulating arbuscule degeneration, possibly as a result of sensing the P_i status of the arbuscule-containing cell (P. Wang *et al.*, 2021). This could occur to avoid cell-autonomous overaccumulation of P_i or alternatively to eliminate inefficient or aged arbuscules that ceased to deliver Pi efficiently. Indeed, transcript levels of two genes associated with arbuscule-collapse *CYSTEINE PROTEASE 3* (*CP3*) and a *CHITINASE* gene (Floss *et al.*, 2017) are overproportionally reduced in *spx1,3* double-mutant roots, as compared to other AM-induced genes (P. Wang *et al.*, 2021; Y. Wang *et al.*, 2021). It is possible that SPX1 and 3 sequester a suppressor of these genes (P. Wang *et al.*, 2021).

Medicago truncatula contains five *SPX* genes, and possibly, different SPX family members have diversified in their ability to interact with other proteins and thereby fulfill different functions. Thus, other SPX family members in *M. truncatula* may fulfill a similar role in regulating AM, as SPX proteins in rice and tomato (Das & Gutjahr, 2022). *M. truncatula* SPX1 and 3 interact with MtPHR2, which is encoded by an ortholog of *L. japonicus PHR1c*, the role of which has not yet been genetically addressed in contrast to *LjPHR1a* (Das *et al.*, 2022). It will be interesting to understand the function of MtPHR2 in AM symbiosis and whether this function is conserved among all PHR family members. It appears unlikely that MtPHR2 is directly involved in arbuscule degeneration. The dual role of SPXs in arbuscule development may be explained by the local increase of P_i in arbuscule-containing cells over time: At the onset of arbuscule development, lack of P_i enables the action of PHR, which induces expression of *PT4* and its orthologues. This leads to locally increased P_i levels by increased P_i uptake via the arbuscule which, in turn, activates SPX proteins that inhibit the action of PHR and thereby lead to reduced expression of arbuscule-containing cell-specific P_i transporter genes and also increased expression of genes related to arbuscule collapse such as *CP3* and *CHITINASE*. However, the *MYB1* gene, which encodes a regulator of arbuscule collapse in the absence of P_i delivery (Floss *et al.*, 2017), is not overproportionally affected by the *spx1,3* mutations (P. Wang *et al.*, 2021). It will be interesting to understand whether it is involved in SPX1 and SPX3-mediated regulation of arbuscule turnover or acts in parallel. Since a role of the PSR system in regulation of the proportion of mature and

degenerate arbuscules was so far only observed in *Medicago*, it will be of interest to address this also in other species.

2. Regulation of P_i transporter genes during AM symbiosis

Upon root colonization by AM fungi, the uptake route for P_i switches from the direct to the mycorrhizal uptake pathway. This is associated with the reduction of the expression of genes encoding for transporters of the direct P_i uptake pathway and an activation of P_i transporter genes expressed in arbuscule-containing cells (Rausch *et al.*, 2001; Harrison *et al.*, 2002; Paszkowski *et al.*, 2002; Karandashov *et al.*, 2004; Yang *et al.*, 2012). Mutants of these AM-specific transporters are impaired in AM symbiosis, resulting from an increased arbuscule turnover (Javot *et al.*, 2007; Yang *et al.*, 2012). This is most likely an effect of the plant's inability to receive P_i from the fungus, confirmed by the accumulation of poly-P_i in arbuscules in *Mtpt4* mutant roots (Javot *et al.*, 2007). Importantly, it indicates that fungal P_i delivery is required for arbuscule maintenance. In *Medicago*, MYB1 was demonstrated to be a transcriptional regulator of the enhanced arbuscule turnover occurring in *pt4* mutants, by inducing the expression of genes encoding hydrolytic enzyme in arbuscule-containing cells (Floss *et al.*, 2017). Analysis of the regulatory regions of the arbuscule-specific P_i transporter genes of plants revealed the presence of a variety of *cis*-regulatory elements (Karandashov *et al.*, 2004). Among them, the CTTC/MYCS and the *P1BS* motif are often found in close association in proximity to the transcriptional start sites (Karandashov *et al.*, 2004; Chen *et al.*, 2011; Lota *et al.*, 2013). While the *P1BS* motif was already known to be bound by PHRs (Bustos *et al.*, 2010), the CTTC/MYCS was later shown to be a binding site of CTTC MOTIF-BINDING TRANSCRIPTION FACTOR 1 (CBX1), an AM-induced WRINKLED (WRI) TF of the AP2 superfamily (L. Xue *et al.*, 2018). The close association of these motifs (Chen *et al.*, 2011; Lota *et al.*, 2013) makes it tempting to speculate that PHRs and CBX1 might act in concert, to regulate target gene expression. Alternatively, PHRs may be involved in opening the chromatin (Barragán-Rosillo *et al.*, 2021) at genomic loci containing AM-relevant genes at low P_i conditions, while CBX1 may afterward induce these genes upon root colonization by AM fungi.

It remains an intriguing open question how PHRs induce expression of arbuscule-containing cell-specific P_i transporter genes upon P_i starvation, while P_i transporter genes of the direct Pi uptake pathway in the rhizodermis show a strong reduction in expression upon AM, although they are normally also activated by PHRs. Are specific transcriptional repressors activated in the rhizodermis during AM colonization to suppress the expression of Pi transporter genes of the direct uptake pathway, or is PHR activity restricted to certain tissues during AM? It will be highly interesting and relevant, to solve these questions in future research. Interestingly, in tomato, it was found that mutation of *SPX1* leads to increased direct P_i uptake and AM colonization at the same time (Liao *et al.*, 2022). This strengthens the hypothesis that additional or PSR-independent factors are involved in the switch between direct and indirect P_i-uptake pathway, since the PSR system seems to be in charge of regulating both in parallel. However, job-sharing between different SPX family members also remains a possibility.

IV. The role of the PSR in other beneficial plant–fungal associations

Besides AM symbiosis, also the symbiosis with Mucoromycotina fine root endophytes (Hoysted *et al.*, 2023) and in woody plants with ectomycorrhizal fungi (Bücking & Heyser, 2003; Cairney, 2011) contributes to the P_i nutrition. Yet, only little is known about the molecular programs that enable these associations (Garcia *et al.*, 2015) and no evidence for the involvement of the plant PSR system in the regulation of associations with fine root endophytes or ectomycorrhizal fungi has been described yet.

However, other fungi benefit AM-incompetent plants (such as *Arabidopsis*) with P_i transfer. It appears that AM-incompetent species may have acquired alternative ways to acquire P_i whether through enhancing the direct uptake pathway, for example, via secretion of organic acids and production of cluster roots (Lambers *et al.*, 2006) or through interaction with alternative fungi (Werner *et al.*, 2018; Almario *et al.*, 2022).

1. Interactions with *Colletotrichum* species

One example is the interaction between *Arabidopsis thaliana* and the fungus *Colletotrichum tofieldiae*. This fungus belongs to a genus comprising majorly pathogens and was shown to form an endophytic association with *Arabidopsis* (Hiruma *et al.*, 2016). It promotes *Arabidopsis* growth under P_i-limiting conditions through transfer of P_i to the plant. This growth promotion requires the main regulators of PSR, PHR1, and PHR1-LIKE (PHL1). Trp-derived indole glucosinolates (IGs) are needed to restrict the fungus, which otherwise over-proliferates, leading to plant growth depression (Hiruma *et al.*, 2016). In a subsequent study, the growth-promoting effect of *C. tofieldiae* on *Arabidopsis* under P_i deficiency coincided with the repression of genes involved in PSR and was associated with increased auxin signaling and promotion of root growth, caused by *C. tofieldiae* colonization at low P_i (Frerigmann *et al.*, 2021); however, it is yet unclear whether the amount of auxin in the root or auxin signaling was increased and whether a possible increase in auxin was caused by plant or fungal biosynthesis.

Genomic and transcriptomic comparisons between the endophytic *C. tofieldiae* and the pathogenic sister species *C. incanum* revealed a decreased set of putative secreted effector genes, an increased number of genes involved in secondary metabolite synthesis, and reduced expression of pathogenicity-related genes in the endophyte (Hacquard *et al.*, 2016). Furthermore, the immune response to *C. tofieldiae* was repressed in *Arabidopsis* roots under P_i starvation, but not under P_i-sufficient conditions. The authors hypothesized that the connectivity between P_i sensing and innate immunity combined with the small genomic changes in *C. tofieldiae* enabled a P_i-status-dependent beneficial interaction between *Arabidopsis* and this fungus (Hacquard *et al.*, 2016).

The major transcriptional regulators of genes involved in biosynthesis of IG precursors are MYB34, MYB51, and MYB122 (Frerigmann & Gigolashvili, 2014). However, a *myb34,21,122* triple mutant affected *Arabidopsis* growth in the presence of *C. tofieldiae* less dramatically than a *cyp79b2b3* biosynthetic mutant, indicating the presence of additional regulatory players of IG biosynthesis. Nevertheless, the presence of *P1BS* elements in the promoters of *MYB34* and *MYB122* and the upregulation of MYB34 expression upon P_i starvation (Barragán-Rosillo *et al.*, 2021) as well as the *PHR1*-dependent increase of glucosinolates upon P_i starvation in *Arabidopsis* (Pant *et al.*, 2015a, b) suggests a direct regulation of IG precursor biosynthesis by PHR1.

Plant growth promotion by *C. tofieldiae* was also observed in tomato and maize, although the mechanism of growth promotion remained unclear (Díaz-González *et al.*, 2020). Further, the question remains how non-Brassicaceae plants that should be devoid of IG biosynthetic enzymes are capable of maintaining a beneficial relationship with *C. tofieldiae*. It is likely that different plants use a variety of chemicals for controling colonizing fungi.

In a different interaction between *Arabidopsis* and the bacterium *Bacillus amyloliquefaciens* strain GB03, the P_i-dependent switch between plant defense response and beneficial relationship was dependent on the bacterial volatile compound diacetyl (Morcillo *et al.*, 2020). It is conceivable that plant and microbial small molecules regulate a large array of interactions between plants and facultatively beneficial microbes, and it will be exciting to learn in the future which molecules influence the promotion of plant Pi uptake by microbes in other plant–microbe interactions.

2. Interactions with *Serendipita indica*

Serendipita indica is another fungal endophyte known to promote plant growth. Root colonization with this fungus results in an increased P_i content in different plant species such as rapeseed, wheat, and soybean (Bajaj *et al.*, 2018; Taghinasab *et al.*, 2018; Wu *et al.*, 2018). While it was assumed earlier, that similar to AM, a direct transfer of P_i from the substrate to the plant occurs via *S. indica* (Yadav *et al.*, 2010, retracted), newer results suggest that colonization with *S. indica* stimulates the expression of genes coding for proteins involved in PSR and P_i homeostasis, such as P_i uptake transporters and phosphatases (Bakshi *et al.*, 2017; Bajaj *et al.*, 2018; Wu *et al.*, 2018). Also, promotion of lateral root growth and root hair length and density by *S. indica* has been observed under P_i-limiting conditions (Bakshi *et al.*, 2015), which may contribute to increased direct P_i uptake. Whether the PHR-SPX system plays a role in the interaction between plants and *S. indica* remains unknown. However, WRKY6, a TF involved in negative regulation of P_i starvation genes and physiological responses (Chen *et al.*, 2009; Stetter *et al.*, 2017; Ye *et al.*, 2018), negatively regulates growth promotion by *S. indica* (Bakshi *et al.*, 2015). These findings suggest that *S. indica* indeed stimulates plant growth by activating certain PSRs (Bakshi *et al.*, 2017; Bajaj *et al.*, 2018; Wu *et al.*, 2018).

V. The role of the PSR system in root nodule symbiosis

Root nodule (RN) symbioses between legumes and nitrogen-fixing rhizobia bacteria are also regulated by the P_i status of the plant (O'Hara, 2001). In fact, nodules seem to require large amounts of Pi. For example, soybean nodules were described as the P_i richest organ of the plant (Sa & Israel, 1991). To establish the symbiosis,

rhizobia enter the plant root via tubular infection threads through root hairs (Parniske, 2018). This structure guides the bacteria into the root cortex, where an additional genetic program induces development of new root organs, the nodules (Popp & Ott, 2011; Suzaki *et al.*, 2015). Inside cells of these organs, the bacteria are taken up into plant-membrane-surrounded symbiosomes, where they differentiate into nitrogen-fixing bacteroides (Haag *et al.*, 2013; Emerich & Krishnan, 2014).

1. The role of the plant P_i status in nodulation

The genetic programs involved in the processes of infection and nodule formation originated during evolution from the common symbiosis signaling network required for the formation of AM and part of the genes are required for both symbioses (Kistner & Parniske, 2002). In rice and *L. japonicus*, it was demonstrated that some common symbiosis genes (e.g. *SYMRK, CCaMK,* and *CYCLOPS*) are targets of PHR2 (Das *et al.*, 2022), which would imply that nodulation is also promoted at low P_i conditions. However, in soybean, P_i deficiency already hampers the earliest physiological responses to rhizobia such as root hair curling, bacterial attachment, and IT formation (Isidra-Arellano *et al.*, 2018), which are processes regulated by SYMRK, CCaMK, and CYCLOPS (Stracke *et al.*, 2002; Yano *et al.*, 2008; Shimoda *et al.*, 2012). This makes it tempting to speculate that the expression of SYMRK, CCaMK, and CYLOPS can also be activated by nitrogen starvation, which is the permissive condition for root nodule symbiosis, but only at sufficient P_i supply. This would be in line with the observation that AM colonization at high P_i conditions can be rescued by low N supply (Nouri *et al.*, 2014) and implies a complex cross talk between P_i and N signaling in the regulation of symbiosis genes, the outcome of which depends on the $P_i:N$ ratio. In fact, it was shown that phosphate starvation responses are controlled by nitrate (Hu *et al.*, 2019; Medici *et al.*, 2019), and it is possible that an opposite scenario also exists.

In soybean, 35 *PHR* homologs were identified, of which six were upregulated in nodules (Xue *et al.*, 2017). These are thought to regulate a whole set of genes upon P_i deficiency that are involved in promoting P_i uptake specifically in nodules (Fig. 3). Among them are transporter-encoding genes like *GmPT5*, which is thought to shuttle P_i from the root tissue toward the nodule (Qin *et al.*, 2012) and *MtPho1.1/1.2* that supports P_i transfer to bacteroides in *Medicago* root nodules (Nguyen *et al.*, 2021). Also, the PSR genes *GmPAP12* and *GmSPX8* play important roles in the regulation of nodule development, nodule number control, and nitrogen fixation, via the control of nodular P_i homeostasis (Xu *et al.*, 2018; Y. Wang *et al.*, 2020). Overexpression of *GmPHT1;11*, a gene usually expressed in nonfixing tissues of the nodule, or the genes encoding its positive regulators *GmPHR1* and *GmPHR4* increased the P_i content, the size of nodules, and the nitrogenase activity per nodule weight, but decreased the nodule number at the same time at both high and low P_i conditions (Lu *et al.*, 2020). This raises the question whether it is favorable to form less but bigger nodules, which contain more P_i per nodule to fuel bacterial N_2 fixation, especially under low P_i conditions.

2. The plant P_i status influences the autoregulation of nodulation

Autoregulation of nodulation (AON) describes a negative regulatory system of plants, which controls nodule numbers and acts by systemic shoot–root signaling. Genes involved in AON, encoding NODULE INCEPTION (NIN), CLAVATA3 (CLV3)/ENDOSPERM SURROUNDING REGION (ESR)-related (CLE) peptides called RICH IN CYSTEINE 1 and 2 (RIC1 and RIC2) and the F-box protein TOO MUCH LOVE (TML), are upregulated under P_i starvation in common bean, and it was hypothesized that they might be under direct control of PHR1 and its homologs because their promoter regions contain *P1BS* elements and their expression is reduced in *PHR*-RNAi lines (Isidra-Arellano *et al.*, 2018, 2020; Ma & Chen, 2021; Okuma & Kawaguchi, 2021). In addition, nodulation of the hypernodulating *nodule autoregulation receptor kinase* (*nark*) mutants, deficient in AON, nodule number was not affected by the plant P_i level (Isidra-Arellano *et al.*, 2020). Thus, the AON system may be the driver of reduced nodule numbers under P_i as shown in soybean (Chaudhary *et al.*, 2008; Hernández *et al.*, 2009; Y. Xue *et al.*, 2018).

VI. The role of the PSR system in plant immunity

Besides beneficial interactions, the PSR system influences the interaction between plants and pathogenic microbes. While the plant aims at sequestering nutrients in order to prevent their accessibility to pathogens (Radtke & O'Riordan, 2006; Hood & Skaar, 2012), pathogens probably aim at manipulating the plant to enable access to nutrients in host tissues. In both scenarios, the PSR system can be a major player.

1. A microbial effector manipulates the PSR system

A high plant P_i status is beneficial to the success of pathogens. Rice leaves grown under high P_i were more susceptible to the pathogenic fungus *Magnaporthe oryzae* than leaves of plants grown under low or sufficient P_i conditions (Campos-Soriano *et al.*, 2020). Increasing P_i levels in plant host cells could thus be a major goal for pathogenic microbes. In line with this idea, it has been observed that infection with *Candidatus liberibacter asiaticus*, the causative agent of the huanglongbing disease in citrus plants, causes specific upregulation of miR399, a key player in the positive regulation of P_i uptake (Zhao *et al.*, 2013). Also, the overexpression of the effector protein SAP11$_{AYWB}$ from parasitic phytoplasma bacteria caused, among other disease symptoms, a strong activation of PSR genes in *A. thaliana*, including *IPS*s, *SPX*s, *PHT*s, and miRNAs involved in PSR, P_i homeostasis, and auxin signaling like miR160, miR396, miR399, miR827, and miR2111 (Lu *et al.*, 2014). Since most of the genes induced by SAP11$_{AYWB}$ are direct targets of PHR1, it seems likely that the effector SAP11 alters PHR1 activity. A possible scenario could be an interaction of both proteins to render PHR1 resistant to negative regulation through sumoylation or interaction with SPX proteins, thereby allowing constitutive nuclear localization, dimerization, and transcriptional activity by PHR1.
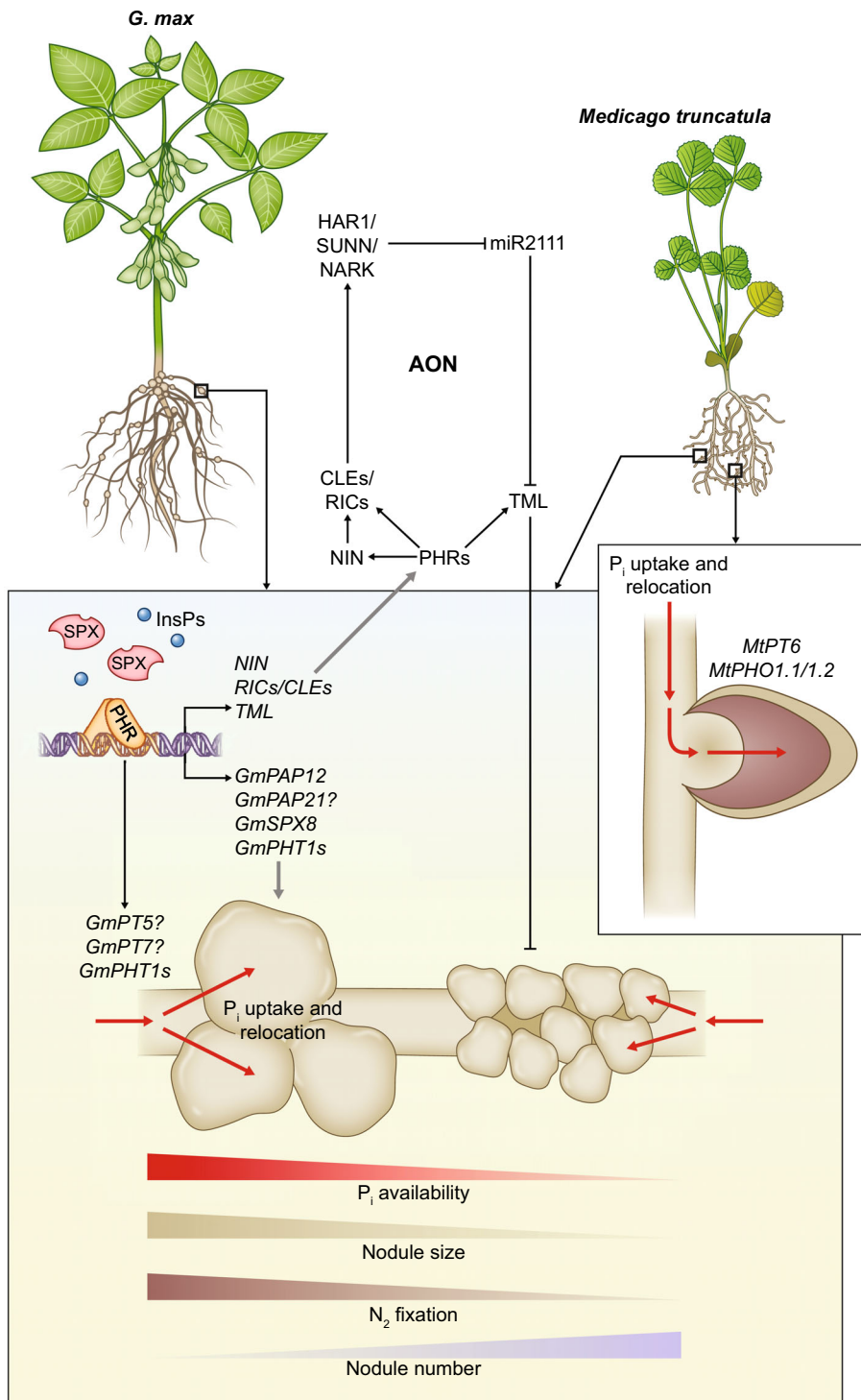
Fig. 3 Regulation of root nodule symbiosis and its adaptation to different P_i levels by the plant phosphate starvation response (PSR) system, as it was described in *G. max* and *Medicago truncatula*. Low P_i conditions cause the formation of numerous small nodules with low N_2 fixation potential. To enable more efficient root nodule symbiosis, PHOSPHATE STARVATION RESPONSE (PHR) transcription factors of leguminous plants induce genes of the autoregulation of nodulation (AON) pathway to restrict nodule number (Isidra-Arellano *et al.*, 2018, 2020), probably to focus the available P_i in fewer, but therefore more efficient nodules. In parallel, genes that promote P_i uptake (*GmPTs*), availability (*GmPAPs*, *GmSPX8*) and relocation (*GmPTs*, *GmPHT1s* in *G. max*; *MtPT6*, *MtPHO1.1/1.2* in *M. truncatula*) are induced by PHRs and promote nodule growth and N_2 fixation (Qin *et al.*, 2012; Li *et al.*, 2017; Xu *et al.*, 2018; Chen *et al.*, 2019; Lu *et al.*, 2020; Y. Wang *et al.*, 2020; Cao *et al.*, 2021; Nguyen *et al.*, 2021). CLE, CLAVATA3/Embryo Surrounding Region-Related; HAR1, Hypernodulation Aberrant Root Formation 1; NARK, nodule autoregulation receptor kinase; NIN, Nodulation Inception; PAP, purple acid phosphatase; PHO1, phosphate 1; PHT, high affinity phosphate transporter; PT, Phosphate Transporter; SUNN, SUPER NUMERIC NODULES; TML, TOO MUCH LOVE.

2. Modulation of plant immunity through the plant P_i status and the PSR system

The surfaces and the interior of plant organs are colonized by diverse bacterial communities. The structure and composition of such communities is influenced by the plant P_i status. Various *Arabidopsis* mutants with defects in PSR pathway components revealed a shift in the composition and structure of the bacterial root endophyte microbiome as compared to WT, when they were grown in the same type of nonsterile top soil, or grown in the presence of a predefined synthetic community (SynCom; Castrillo *et al.*, 2017). Interestingly, application of the SynCom negatively affected P_i accumulation in shoots of WT plants under P_i depletion but not under P_i-replete conditions, suggesting that bacteria in the SynCom may compete with the plant for P_i under P_i starvation conditions. Comparative transcriptomics of WT and *phr1* (and

phr1 phl1) mutants in the presence of the SynCom revealed a strong enrichment of genes involved in plant immunity, including an upregulation of salicylic acid and a downregulation of jasmonic acid signaling markers in *phr1* (and *phr1 phl1*) mutants. ChIP-Seq confirmed that at least some of them are direct targets of PHR1. Interestingly, *phr1 phl1* mutants also showed a stronger induction of defense genes in response to bacterial flagellin, indicating that *phr* mutants defend more strongly against certain bacteria as compared to the WT. Congruously, the *phr1 phl1* double mutant of *A. thaliana* displayed enhanced resistance against the bacterial pathogen *Pseudomonas syringae* and also the oomycete *Hyaloperonospora arabidopsidis* (Castrillo *et al.*, 2017). This links plant immunity to PSR, with PHR1 as common regulator, and likely explains the change in root bacterial communities when PSR regulators are mutated (Castrillo *et al.*, 2017). In addition to immunity modulation, the PSR system may affect rhizosphere microbiota through altering the chemical cocktail of root exudates in order to attract beneficial microbes (Rolfe *et al.*, 2019). The shifts in microbial communities via the PSR system across the whole plant were confirmed in a later laboratory study and dynamics in root-inhabiting communities as a consequence of changes in P_i levels were also observed for fungal microbiota in the field (Yu *et al.*, 2018; Fabiańska *et al.*, 2019; Finkel *et al.*, 2019).

PHR1 directly activates genes encoding RAPID ALKALINIZATION FACTORS (RALFs, Fig. 4), which interfere with defense activation (Tang *et al.*, 2022). RALFs bind to their receptor FERONIA, which then inhibits complex formation of the flg22 receptor FLAGELLING INSENSITIVE 2 (FLS2) with its co-receptor BRASSINOSTEROID ASSOCIATED KINASE 1 (BAK1; Stegmann *et al.*, 2017; Tang *et al.*, 2022). This may allow the recruitment of beneficial bacteria, such as *Pseudomonas* and *Bacillus* species to the roots, to alleviate P_i starvation (Tang *et al.*, 2022), as it was hypothesized earlier (Castrillo *et al.*, 2017; Finkel *et al.*, 2019). Furthermore, the interaction with beneficial fungi, such as *C. tofieldiae, S. indica*, and especially AM fungi, which are also suppressed by SA (Blilou *et al.*, 1999) is likely promoted by a reduced immune status of the plant. This raises the question of how plants can afford to reduce their immune status to promote beneficial microbes while avoiding simultaneously to be overrun by detrimental ones. Interestingly, further experiments with SynComs revealed that upon P_i starvation numerous neutral or beneficial microbes interacting with plants showed an opportunistic character and changed toward exploitation of their plant host (Finkel *et al.*, 2019). Is the plant immune status again fostered when beneficial microbes have increased the P_i status of the plant? If this would be the case, how would the beneficial interaction be maintained? One solution could be very tight and situation-dependent regulation. Indeed, in roots of *Arabidopsis* seedlings defense is tightly and spatially regulated; and immune receptor genes are expressed only in vulnerable sites of the root and induced only by co-occurrence of microbe-associated molecular patterns (MAMPs) and wounding (Zhou *et al.*, 2020). In addition, it is possible that immune response induction is avoided or locally suppressed in cells colonized by beneficial microbes possibly through the action of microbial effectors or the delivered goods (e.g. P_i). Some microbial structures like AM fungal arbuscules are turned over after a short live-time of about 2–3 d (Kobae & Hata, 2010). It would be interesting to investigate whether this may be caused by a plant cell-autonomous increase in defense caused by P_i accumulation in the arbuscule-containing cell.

In pathogenic binary plant–microbe interactions, increased P_i content caused by loss-of-function of the ubiquitin ligase NLA that is, like PHO2, a negative regulator of P_i uptake (Lin *et al.*, 2013a), led to increased resistance against the fungal pathogen *Plectosphaerella cucumerina* in *Arabidopsis* (Val-Torregrosa *et al.*, 2022). This was probably due to increased camalexin, salicylic acid, and jasmonic acid content in *nla* plants (Val-Torregrosa *et al.*, 2022). In a similar case, the loss-of-function of InsP kinases INOSITOL PENTAKISPHOSPHATE 2-KINASE 1 (IPK1) and INOSITOL 1,3,4-TRISPHOSPHATE 5/6-KINASE 1 (IPTK1), which leads to higher accumulation of free P_i, caused increased defense against bacterial pathogens (Gulabani *et al.*, 2022). Another study showed instead that mutation of *phr1* increased susceptibility toward a pathogenic oomycete *Phytophthora cinnamoni* (Eshraghi *et al.*, 2014). The authors suggested that this could be mediated by auxin signaling as mutation of the gene encoding the auxin receptor component TIR1 also displayed enhanced susceptibility (Eshraghi *et al.*, 2014).

The observation that cellular P_i levels can have positive or negative effects on success and growth of pathogens may be explained by a specific balance between increased availability of P_i for the pathogen and reduced PSR signaling, leading to enhancement of a subset of immune responses, which may be effective against some but not all microbes. The combination of the overall physiological and metabolic status of the plant and the arsenal of virulence factors of the microbe likely impacts this balance.

3. Defense regulators modulate P_i uptake

Immunity and the PSR system seem to affect each other reciprocally as factors involved in plant immunity influence P_i homeostasis. The *Arabidopsis* receptor-like kinase BOTRYTIS INDUCED KINASE 1 (BIK1) that is an important interactor of immune receptors like FLS2 and PEPR (Lu *et al.*, 2010; Laluk *et al.*, 2011; Liu *et al.*, 2013; Lin *et al.*, 2013b) acts as a negative regulator of PSRs (Zhang *et al.*, 2016). The *bik1* mutant shows symptoms of P_i starvation and an increased tissue P_i content At P_i starvation as well as high P_i (Zhang *et al.*, 2016). Upon treatment with MAMPs such as flg22 and elf18, BIK1 acts together with PBS1-LIKE KINASE 1 (PBL1) to phosphorylate the P_i transporter PHT1;4, thereby decreasing P_i transport (as determined through changes of the electronic plasma membrane potential via intracellular microelectrodes inserted into roots hairs) and uptake (Dindas *et al.*, 2022). This supports a direct connection between plant immunity and P_i nutrition via BIK1. However, it is unclear how BIK influences P_i uptake in the absence of elicitation, as suggested by the PSR phenotype of *bik1* (Zhang *et al.*, 2016). Nonelicited *bik1 pbl1* double-mutant root hairs displayed only a very slight decrease in P_i transport activity as compared to WT plants (Dindas *et al.*, 2022). It is possible that *bik1* also influences root-hair-independent P_i uptake or that *pbl1* suppresses the PSR-
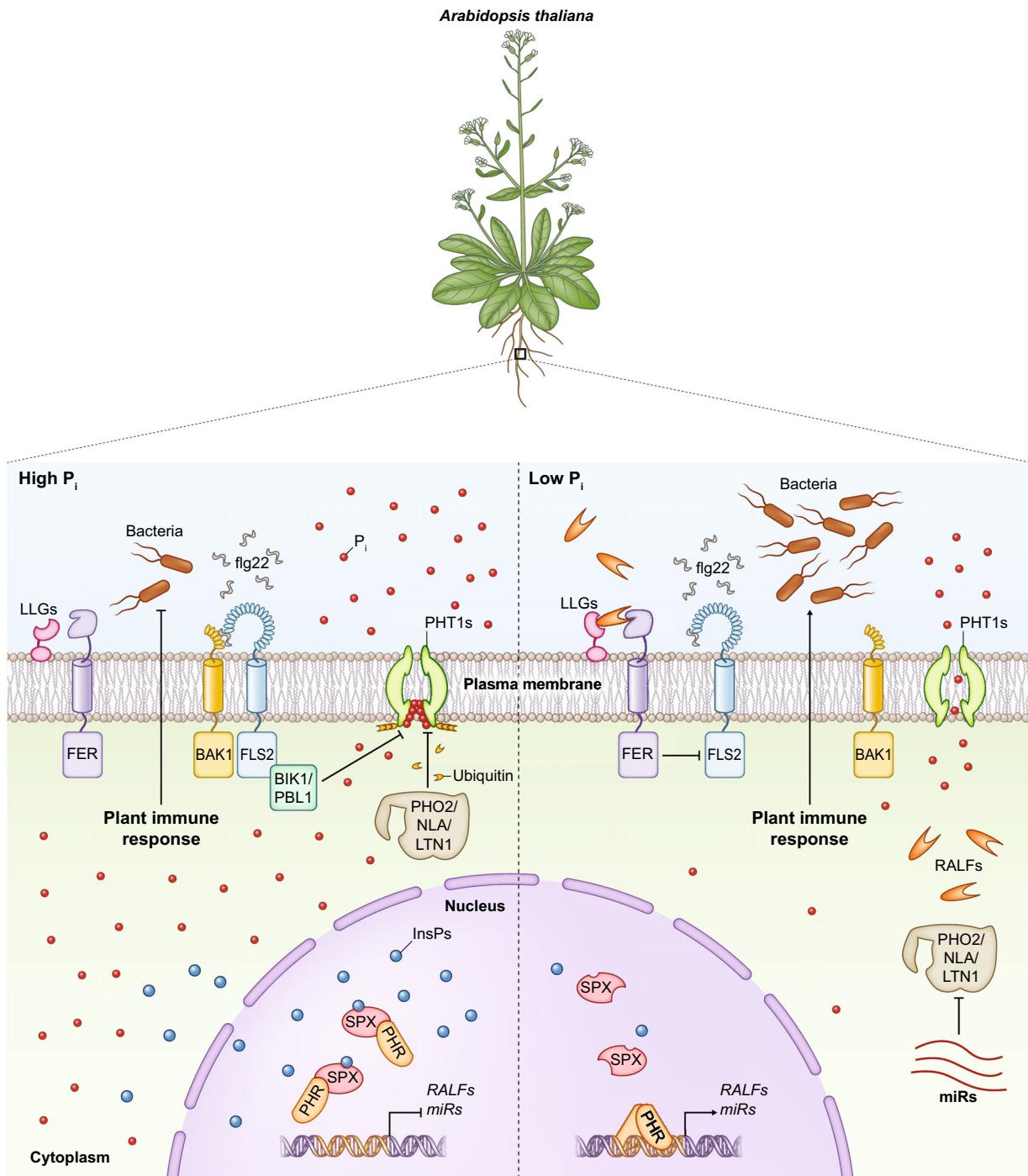
Fig. 4 Plants modulate the community composition of rhizosphere bacteria through P_i-dependent regulation of plant immunity. At high P_i conditions, MAMPs from rhizosphere bacteria such as flg22 promote complex formation of the surface receptor-like kinases like FLAGELLIN SENSING 2 (FLS2) and BRASSINOSTEROID INSENSITIVE 1 (BAK1) leading to an immune response that alters the composition of the bacterial microbiome (Tang *et al.*, 2022). P_i uptake is directly inhibited by the immunity-induced kinases BOTRYTIS INDUCED KINASE 1 (BIK1) and PBS-like 1 (PBL1) that interact with immune receptor complexes and lower the P_i transport activity of epidermally located phosphate transporters (PHT1s) by phosphorylation (Dindas *et al.*, 2022). The abundance of PHT1s is reduced after ubiquitylation by PHOSPHATE 2 (PHO2), LEAF TIP NECROSIS 1 (LTN1), and NITROGEN LIMITATION ADAPTATION (NLA; Hu *et al.*, 2011; Liu *et al.*, 2012; Park *et al.*, 2014; Yue *et al.*, 2017). Upon low P_i conditions, the amount of inositol phosphates (InsPs) becomes insufficient to repress the phosphate starvation response (PSR) system leading to transcriptional activation of miRNAs and genes encoding RAPID ALKALINIZATION FACTORS (RALFs; Fujii *et al.*, 2005; Bari *et al.*, 2006; Gu *et al.*, 2010; Lundmark *et al.*, 2010; Tang *et al.*, 2022). Subsequently to the perception of the RALFs, receptor complexes of FERONIA and LORELEI-LIKE GPI-ANCHORED PROTEINs (LLGs) dampen the activity of FLS2 which reduces the plant immune response, enabling growth of rhizosphere bacteria, possibly to promote P_i uptake (Shen *et al.*, 2017; Tang *et al.*, 2022). P_i uptake at the root surface is increased by active HIGH AFFINITY PHOSPHATE TRANSPORTERS (PHT1s), while the abundance of their ubiquitin-conjugating enzymes is decreased by post-transcriptional silencing via the corresponding miRNAs (Aung *et al.*, 2006; Bari *et al.*, 2006; Chiou *et al.*, 2006; Hu *et al.*, 2011; Lin *et al.*, 2013a).

related *bik1* phenotype in the absence of stimulation by PAMPs. P$_i$ transport remains to be tested in single *bik1* and *pbl1* mutants.

In line with the idea that inhibition of P$_i$ uptake enhances immunity, mutation of *PHT1;4* (and *PHT1;1*) caused a reduced susceptibility to the root pathogenic bacterium *Ralstonia solanacearum* (Dindas *et al.*, 2022). This appears counterintuitive, as defense against *R. solanacearum* relies on SA signaling (Nakano & Mukaihara, 2018), which was observed to be reduced upon P$_i$ starvation, while JA-signaling that favors pathogenicity of *R. solanacearum* increased upon P$_i$ starvation (Castrillo *et al.*, 2017; Nakano & Mukaihara, 2018). However, it is possible that the low P$_i$ content of the plant tissue directly influences *R. solanacearum*'s success or that other effects of Pi starvation influence pathogenicity. Furthermore, *pht1;1 pht1;4* mutants displayed changes in microbiome composition, which to a small extent resembled those of *bik1* (Dindas *et al.*, 2022). Unfortunately, *bik1* and *pht1;1 pht1;4* were in different genetic backgrounds (Dindas *et al.*, 2022), preventing a direct comparison of microbiome assemblies. It is possible that BIK1 and PBL1 suppress P$_i$ uptake to enhance immunity. An alternative hypothesis could be that P$_i$ import is limited to conserve ATP for defense responses.

VII. Conclusion and outlook

Although our knowledge is still fragmentary, it is now emerging that the PSR system controls the outcome of plant–microbe interactions in accordance with the plant phosphate status. It is directly wired to genetic programs regulating symbioses and immune responses, and at low phosphate conditions, PHR TFs directly activate genes required for AM and repress genes involved in nodule formation and in defense. Thereby, the PSR system is a major player in shaping the microbial community interacting with a given plant. It will now be fascinating to uncover when the PSR system evolved, at which position in the algal or plant phylogeny it was wired to pathways regulating plant–microbe interactions and which role this may have played in plant–microbe co-evolution. It will be interesting to understand how the switch of the P$_i$ uptake route during AM is regulated, although both routes require the PSR system for their activation. We need information about how plants employ the PSR system to suppress immune responses to facilitate interaction with beneficial microbes, while still protecting themselves against pathogens; and which molecules and mechanisms steer 'behavioral' switches of facultatively beneficial microbes. Moreover, we need to identify and understand the molecular mechanisms regulating plant–microbe interactions in response to the relative abundance of multiple mineral nutrients. Together this knowledge will provide an important basis for innovations for sustainable agricultural practices based on plant breeding, fertilizer formulations, application of beneficial microbial communities, and pathogen management.

Competing interests

None declared.

Author contributions

MP designed the outline of the review, designed and drew the figures with input from CG. MP and CG wrote the review.

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