OPEN BIOLOGY

rsob.royalsocietypublishing.org

Review

Cite this article: Liu N, Shang W, Li C, Jia L, Wang X, Xing G, Zheng WM. 2018 Evolution of the *SPX* gene family in plants and its role in the response mechanism to phosphorus stress. *Open Biol.* **8**: 170231.
http://dx.doi.org/10.1098/rsob.170231

Received: 9 October 2017
Accepted: 1 December 2017

Subject Area:
molecular biology

Keywords:
plant, *SPX* gene, gene family, P signalling and homeostasis, functional analysis

Author for correspondence:
WenMing Zheng
e-mail: wmzheng@henau.edu.cn

†These authors contributed equally to this work.

THE ROYAL SOCIETY PUBLISHING

Evolution of the *SPX* gene family in plants and its role in the response mechanism to phosphorus stress

Na Liu†, Wenyan Shang†, Chuang Li, Lihua Jia, Xin Wang, Guozhen Xing and WenMing Zheng

State Key Laboratory of Wheat and Maize Crop Science, Collaborative Innovation Center of Henan Grain Crops, College of Life Sciences, Henan Agricultural University, Zhengzhou 450002, People's Republic of China

NL, 0000-0003-0382-6345; WMZ, 0000-0002-7709-2164

Molecular and genomic studies have shown the presence of a large number of *SPX* gene family members in plants, some of which have been proved to act in P signalling and homeostasis. In this study, the molecular and evolutionary characteristics of the *SPX* gene family in plants were comprehensively analysed, and the mechanisms underlying the function of *SPX* genes in P signalling and homeostasis in the model plant species *Arabidopsis* (*Arabidopsis thaliana*) and rice (*Oryza sativa*), and in important crops, including wheat (*Triticum aestivum*), soya beans (*Glycine max*) and rapeseed (*Brassica napus*), were described. Emerging findings on the involvement of *SPX* genes in other important processes (i.e. disease resistance, iron deficiency response, low oxygen response and phytochrome-mediated light signalling) were also highlighted. The available data suggest that *SPX* genes are important regulators in the P signalling network, and may be valuable targets for enhancing crop tolerance to low P stress. Further studies on SPX proteins should include more diverse members, which may reveal SPX proteins as important regulatory hubs for multiple processes including P signalling and homeostasis in plants.

1. Introduction

The *SPX* family was named after *SYG1*, *PHO81* and *Xpr1*, the first three *SPX* gene members identified [1]. *SYG1* and *PHO81* encode yeast *gpa1* suppressor and cyclin-dependent kinase, respectively, while *Xpr1* codes for the xenotropic and polytropic retrovirus receptor 1 in humans. More and more studies have shown that *SPX* genes are involved in phosphorus (P) signalling and homeostasis and are prevalent in plants, and phosphate transport is impaired if the SPX domain is mutated [2]. P is an indispensable macroelement required for normal plant growth and development, and P content is quite high in plant tissues. P is not only an important component of membranes and nucleic acids, but also plays important roles in diverse physiological processes, including photosynthesis, enzyme activity regulation, respiration, signal transduction, oxidation–reduction reactions, energy metabolism and carbon metabolism [3–6]. Plants absorb P mainly from the soil through their roots [7,8]. Soil P exists primarily in the forms of calcium, iron and aluminium salts, and organic molecules, which are difficult for the roots to absorb [9]. This decreases the bioavailability of P, leading to an available P content in the soil that is far lower than that required for normal plant growth [10–12]. In plant cells, P concentration in the cytosol is approximately 60–80 µM [13], which is much higher than the concentration of available P in the soil (less than 10 µM) [14]. Thus, plants are usually under low P conditions. The phenotypic symptoms of P deficiency are mainly dark-green leaf colour, reduced elongation rate of shoot and decreased leaf size [15]. To improve crop yield in agricultural production, a

large quantity of P fertilizers is often applied to solve the problem of P deficiency. However, this approach can cause not only water eutrophication but also overexploitation and consumption of phosphate ore, which is non-renewable. Solving this problem is critical to environmental protection and sustainable development. To adapt to P-deficient environments, plants undergo phenotypic changes in the root system to increase the absorption of P from the soil [16–19]. A series of P transport mechanisms at the molecular level are gradually established to overcome P starvation and to maintain P homeostasis, in which phosphate transporter is the basic effector involved in P uptake, transfer and storage [20,21]. These mechanisms are controlled by complex and sophisticated molecular regulatory networks. Recently, many genes have been identified and functionally linked to these molecular regulatory networks, including various protein-encoding genes and non-coding RNA genes [22]. Hence, the discovery of genes conferring low P tolerance has great importance.

In this study, the structural and evolutionary characteristics of the *SPX* gene family in plants were systematically analysed, the latest research progress on *SPX* gene functions was summarized and the mode of action of these genes in the P regulatory signalling network was discussed.

2. Structural characteristics of *SPX* genes and proteins in plants and their relationships with phosphorus metabolism

The SPX domain found in eukaryotic proteins is rather conserved and has hydrophilic properties [23]. It is often located at the N-terminus of eukaryotic proteins [23]. SPX domain has an average length of 165 amino acids and can be divided into three subdomains with 30–40 amino acids in each (figure 1). They are separated from each other by low similarity regions [24]. In plants, SPX domains can be grouped into several distinct subfamilies: SPX proteins carrying only SPX domain, SPX–EXS proteins containing SPX and an EXS (ERD1, XPR1 and SYG1) domain, SPX–MFS proteins with SPX and the major facility superfamily (MFS) domain and SPX–RING proteins containing SPX and the RING-type zinc finger domain [25]. Many studies have shown that the SPX domain is closely related to P signalling and plays an important role in maintaining P homeostasis [22,26–28].

The SPX domain can indicate the phosphate status in fungal, plant and human cells. SPX domain-containing proteins are indispensable for the absorption, transport, storage and signal transduction of inorganic P in eukaryotes. Wild *et al*. [2] studied the ligand of the SPX domain and suggested that the domain provided a binding surface for small molecules (inositol polyphosphate signalling molecules, InsPs). In this way, the balance of P in plant cells can be regulated by the binding of different InsPs to SPX. In phosphate-deficient plant cells, InsPs bind to SPX domains, being able to interact with several other proteins involved in the regulation of P signalling in plants [2,29]. If the SPX domain is mutated, then phosphate transport capacity is impaired [2], highlighting the unique importance of the SPX domain in P metabolism.

P exists in different molecular forms in plants to serve their needs at different times [30]. One form is inorganic polyphosphate (polyP). PolyP includes hundreds of types of phosphoric anhydrides, which can be hydrolysed to meet the needs of various molecular processes [31–33]. In yeast, the vacuolar transporter chaperone (VTC) complex can synthesize polyP [34]. The VTC is a fairly large protein complex (Vtc1–5) located on the vacuole membrane [35,36]. Approximately 80% of VTC proteins contain an SPX domain, which may contribute to P homeostasis in the cells [5]. Additionally, an important role for the phosphate starvation response 1 (PHR1) protein has been identified in the P signalling network. This MYB-like transcription factor is homologous to phosphorus starvation response 1 (PSR1), which participates in the P sensing process in *Chlamydomonas reinhardtii* [37,38]. PHR1 regulates the expression of *AtACP5*, *AtIPS1*, *PHT1.1* and *RNS1* [39,40], as well as the expression of several PSR genes, including *microRNA399* and the *SPX* genes [38], by binding to their promoters through the *cis*-element PHR1-binding sequence (P1BS; GNATATNC) [40–42].

3. Evolutionary analysis of *SPX* gene family in plants

Through analysis of existing plant genomic sequences, 20 *SPX* gene family members have been identified in *Arabidopsis*, including four *SPX* genes whose deduced proteins contain only the SPX domain [26]. Meanwhile, 15 *SPX* gene family members are identified in rice, including six *SPX* genes whose products carry only the SPX domain [24]. Numerous *SPX* gene family members also exist in the genomes of legumes and other important crops [43,44]. A phylogenetic tree was constructed for some of the important *SPX* genes using the multi-sequence alignment generated by ClustalW and the neighbour-joining method with 1000 bootstrap replicates in MEGA software (figure 2). The results showed evolutionary divergence in *SPX* genes, and the compared genes were clustered into five types of sub-structures (numbered I, II, III, IV and V). Most of the *SPX* genes fell into types I and IV, whereas no more than five genes fell into each of the other three types. *GmSPX8*, *GmSPX5*, *OsSPX4*, *OsSPX6* and *TaSPX129* genes exhibited the most rapid evolution for each type. The paralogues of these genes included *AtSPX1*/*AtSPX2*, *GmSPX2*/*GmSPX4*, *GmSPX5*/*GmSPX9*, *OsSPX1*/*OsSPX2*, *GmSPX1*/*GmSPX10* and *OsSPX5*/*OsSPX6*. Each gene may evolve under different evolutionary pressure and may possibly acquire new function during the course of evolution.

A multiple sequence alignment of the SPX domains was analysed (figure 3). The result showed a high degree of similarity in the SPX domains in *Arabidopsis*, rice, common beans, soya beans, wheat and *Brachypodium*. The SPX domains in TaSPX129 and BdSPX129 were of SPX–MFS type, and have an increased length, whereas the remaining SPX domains ranged from 200 to 400 amino acids. Amino acid point mutations are the main sources of variation, which may affect the role of *SPX* genes in P regulatory networks in plants. Notably, the SPX domains in TaSPX129 and BdSPX129 had many insertions (figure 3); whether this is caused by the presence of an additional MFS domain is a matter for further study.

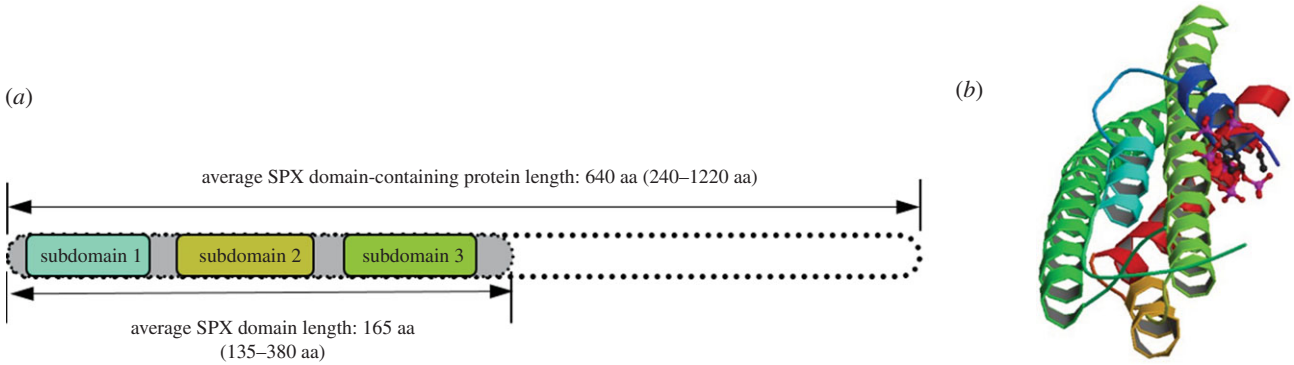

Figure 1. Main structural features of SPX proteins. (*a*) The N-terminal SPX domain can be divided into three well-conserved subdomains separated by low similarity regions, with 30–40 amino acids in each subdomain (Secco *et al*. [24]). (*b*) Crystal structure of the SPX domain in the Vtc4 protein of *Chaetomium thermophilum* in complex with inositol hexakisphosphate (InsP6). Classification: inositol phosphate-binding protein. SPX helical bundles provide a positively charged ligand-binding surface (Wild *et al*. [2]; http://www.rcsb.org/pdb/explore.do?structureId=5IJP).

4. Research progress on the functional analysis of *SPX* genes in plants

A large number of SPX domain-containing proteins have been identified in plants [11]. Here, SPX proteins refer to the proteins that contain only the SPX domain. Owing to their presence in different subcellular structures, they may have different functions in the P signalling network. Here, we summarize the studies on the genes whose proteins contain only the SPX domain, including *SPX1–4* in *Arabidopsis*, *SPX1–6* in rice, *SPX1–10* in soya beans, *SPX1–3* in common bean and *TaSPX129* in wheat (table 1).

4.1. Functional analysis of *SPX* genes in *Arabidopsis*

Twenty SPX domain-containing proteins were found in *Arabidopsis*, among which four proteins contained only the SPX domain (AtSPX1–AtSPX4) [26]. AtSPX1, localized in the nucleus, is a P-dependent suppressor of *AtPHR1* in *Arabidopsis* [53]. *AtPHR1* overexpression results in an increase in P concentration in the shoot and induces the expression of a series of Pi starvation-induced (PSI) genes that encode phosphate transporter, phosphatase or RNase [54,55]. Co-immunoprecipitation experiment showed that the AtSPX1/AtPHR1 interaction was strongly dependent on P level. AtSPX1 is a competitive suppressor that binds AtPHR1 through its recognition sequence. The working model in figure 4 depicts the interactions of AtSPX with AtPHR1 in response to cellular Pi concentration for PSI transcription. Under high P conditions, AtSPX1 has a high binding affinity for AtPHR1, and thus the process by which AtPHR1 regulates PSI genes through the P1BS is inhibited, resulting in a decrease in PSR gene expression. Under P deficiency conditions, the AtSPX1/AtPHR1 interaction weakens, thus facilitating the binding of AtPHR1 to the P1BS to regulate PSR gene expression [49].

In *Arabidopsis*, no significant phenotypic differences have been found among the single-gene knockout mutants of *Atspx1*, *Atspx2* and *Atspx4* under P-sufficient or -starvation conditions. However, in plants with *AtSPX1* overexpression, the expression levels of some PSI genes (i.e. *ACP5*, *PAP2* and *RNS1*) are significantly increased regardless of P concentration, thus suggesting that *AtSPX1* may function in the transcriptional regulation of P starvation. Additionally, inhibition of *AtSPX3* through RNAi can change the phenotypes and gene expression levels under P-starvation conditions, rendering an increase in P concentration in the shoot tissues and a reduced P concentration in the roots [26,43]. The expression levels of *AtPHT1–4*, *AtPHT1–5*, *AtACP5*, *AtRNS* and *AtAT4* in *spx3* deletion mutants are increased irrespective of P concentration [43], indicating that AtSPX3 is a negative regulator of the signalling process of P starvation. Collectively, these results indicate that SPX proteins have functional redundancy with one another and can serve as an important role in regulating Pi signalling and homeostasis in plants.

4.2. Functional analysis of *SPX* genes in rice

P is an important nutrient element that limits the yield of rice. Studies of P relevant genes in rice, especially *SPX* genes, can potentially aid rice yield improvement. A total of 15 SPX domain-containing proteins have been identified in rice, of which only six are SPX proteins (OsSPX1–OsSPX6) [5]. OsSPX1 inhibits P uptake and P-starvation signalling through negative feedback regulation [27,55]. OsSPX1 is induced by P starvation in the roots, and inhibition of *OsSPX1* by RNAi leads to an excessive accumulation of P and thus induces severe toxicity. This phenotype is similar to that observed in the plants overexpressing *OsPHR2* and the *pho2* mutant. *OsPHR2* overexpression leads to increased PSI gene expression, including *IPS1* and *PT2*, which promotes excessive P absorption and accumulation and results in leaf necrosis. Quantitative polymerase chain reaction (qRT-PCR) assay showed that *OsSPX1* expression was strongly induced in the plants with *OsPHR2* overexpression and the *pho2* mutant, suggesting that *OsSPX1* may function downstream from *PHO2* and *OsPHR2*. Wang *et al*. [27] analysed the expression levels of 10 genes involved in the rice P-starvation signalling pathway. *OsPT2* and *OsPT8* were significantly induced in *OsSPX1* RNAi plants, pointing to increased P transport and accumulation. By contrast, *OsSPX1* overexpression inhibited the expression of 10 phosphate starvation-mediated genes, including *IPS1*, *IPS2*, *OsPAP1*, *OsSQD2* (*sulfo quinovosyl diacylglycerol 2*), *miR399d*, *miR399j*, *OsPT2*, *OsPT3*, *OsPT6* and *OsPT8*. However, in the double mutant plants with overexpression of *OsPHR2* and *OsSPX1*, P concentration and PSR gene expression levels were basically the same as those in wild-type plants, which indicated that

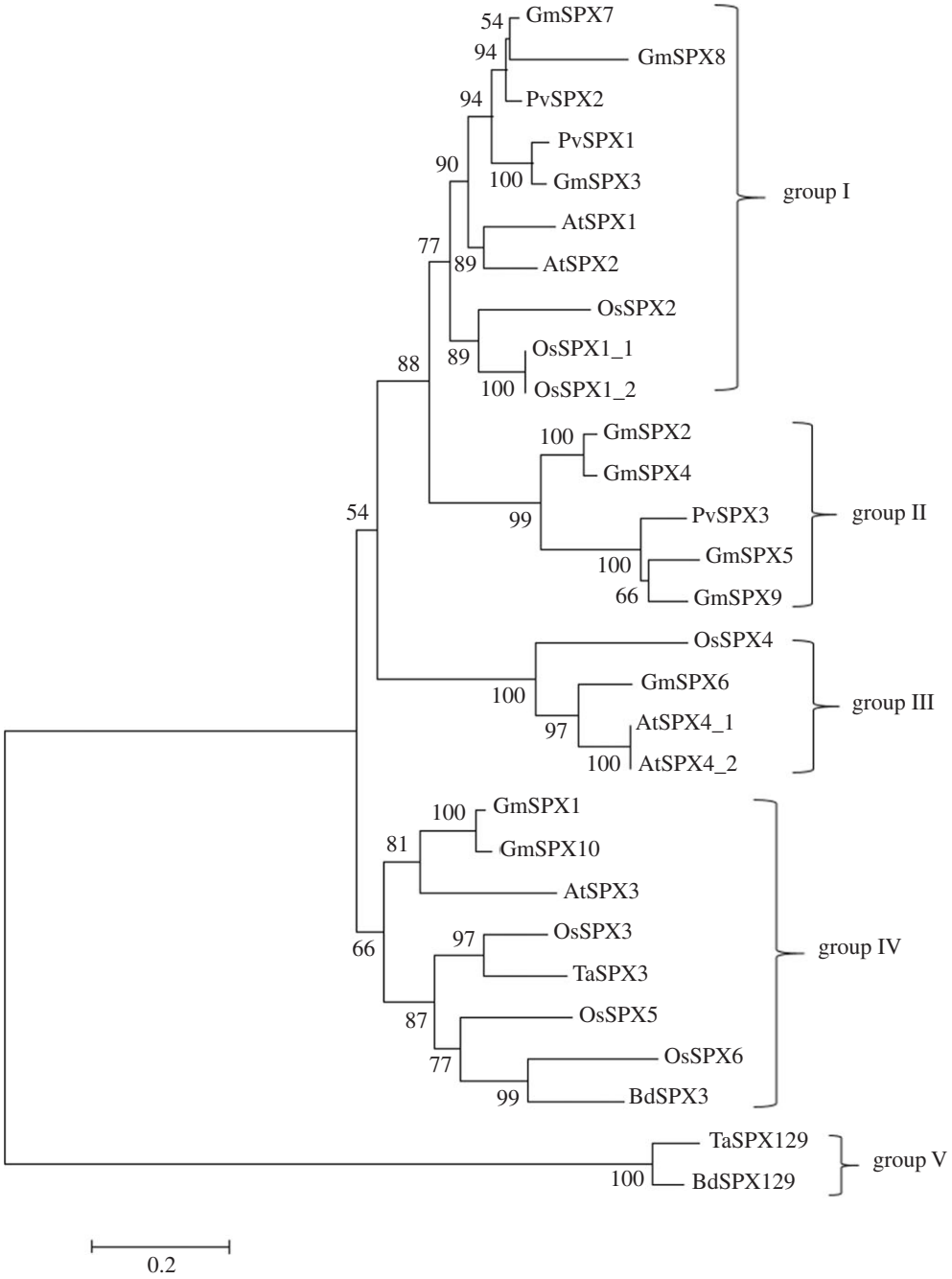

Figure 2. Phylogenetic tree of SPX domain-containing proteins from different plant species. The tree was constructed with the neighbour-joining method by MEGA with 1000 bootstrap replicates. At, *Arabidopsis thaliana*; Os, *Oryza sativa*; Pv, *Phaseolus vulgaris*; Gm, *Glycine max*; Ta, *Triticum aestivum*; Bd, *Brachypodium distachyon*.

OsSPX1 was a negative regulator of OsPHR2-mediated signal transduction. Previous studies found that overexpression of *OsSPX1*, *OsSPX2*, *OsSPX3*, *OsSPX4* and *OsSPX5* attenuated the phenotype of *OsPHR2* overexpression [45–47,53]. Thus, OsSPX1, OsSPX2 and OsSPX4 may interact with OsPHR2 and inhibit its binding to the P1BS *cis*-acting element (figure 4). The interaction between SPX proteins and PHR1/2 was strongly dependent on P concentration [45,47]. Knockout of *OsSPX1*, *OsSPX2* and *OsSPX4* results in P accumulation in the shoot and significant leaf tip necrosis [45,47]. P accumulation and leaf necrosis also occurred in the *Osspx3* and *Osspx5* double mutant, and the expression levels of PSI genes, including *IPS1*, *miR399*, *PT2*, *miR827*, *PAP10* and *SQD2*, were significantly upregulated [46]. This observation indicated that *OsSPX3* and *OsSPX5* were homologous and that they responded to P stress at the transcriptional and post-transcriptional levels. Collectively, the data gathered so far support the function of the studied *OsSPX* genes in rice tolerance to P deficiency by regulating P acquisition and its transport from roots to leaves.

4.3. Functional analysis of *SPX* genes in legumes

Phylogenetic analysis demonstrated that GmSPXs 1–10 can be divided into three groups [43]. Quantitative PCR assay showed that the expression of these genes was significantly increased under low P conditions and decreased rapidly one day after P supplementation [43]. The expression of these genes was highly sensitive to low P conditions. Overexpression of *GmSPX3* led to increased P concentration in both leaf and root tissues, and increased transcriptional levels of seven PSI genes in the root hairs, under high P conditions [44]. Analysis

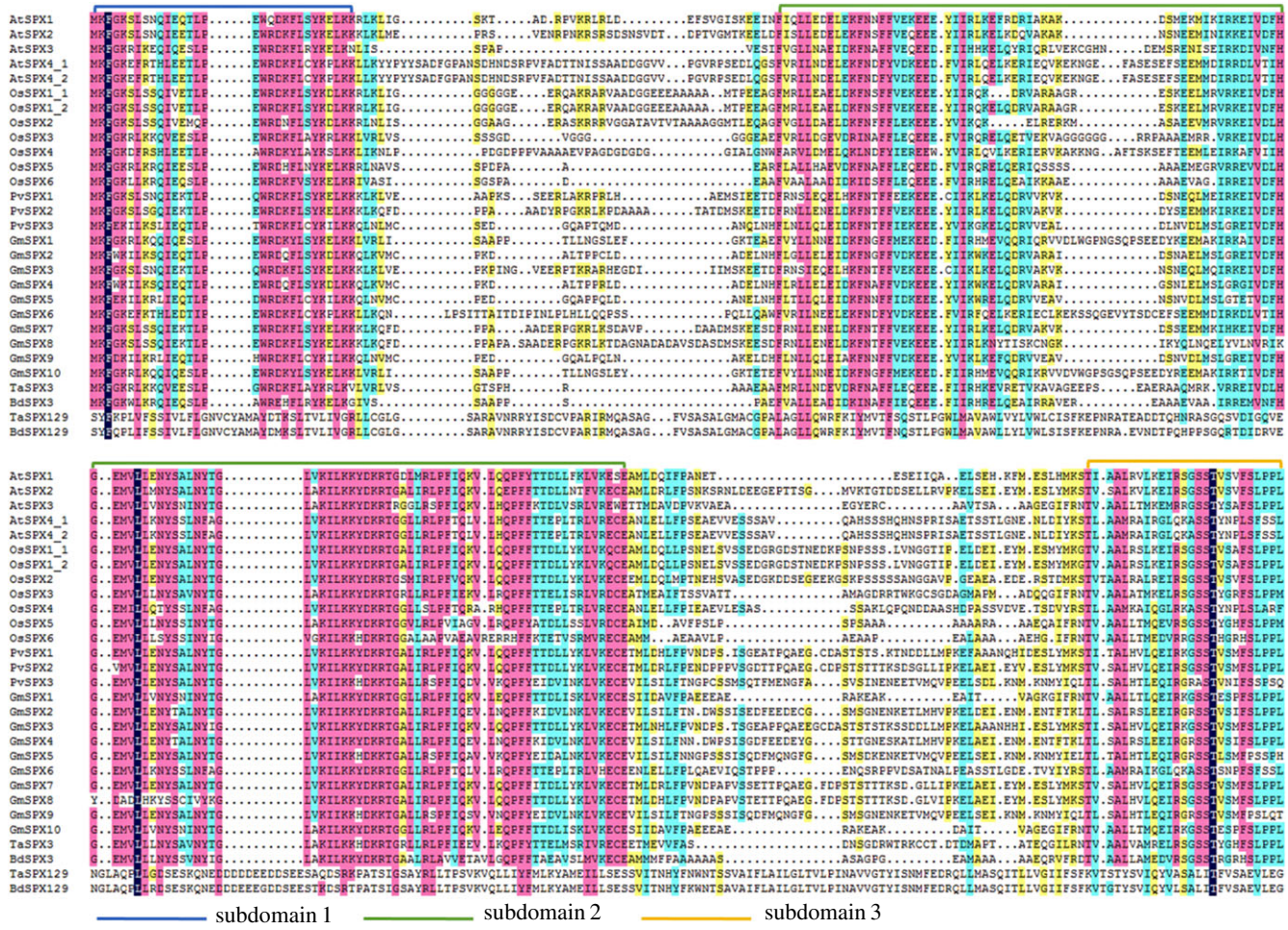

Figure 3. Multiple alignment of the SPX domains in different SPX proteins. The multiple alignment was generated using DNAMAN with different colours representing different homology of amino acids. The three subdomains are distinguished by coloured brackets. At, *Arabidopsis thaliana*; Os, *Oryza sativa*; Pv, *Phaseolus vulgaris*; Gm, *Glycine max*; Ta, *Triticum aestivum*; Bd, *Brachypodium distachyon*.

of *GmSPX1* overexpression in *Arabidopsis spx3* mutant showed that GmSPX1 negatively regulated many PSR genes, including *AtPHT1–4*, *AtPHT1–5*, *AtACP5*, *AtRNS* and *AtAT4*, in a P level-dependent manner [43]. Furthermore, GmSPX1 interacted with a newly identified P starvation-induced transcription factor GmMYB48, and this interaction may represent a potential suppressor module of the P signalling network in soya bean [43]. Three SPX proteins (PvSPX1–PvSPX3) have been found in common bean (*Phaseolus vulgaris*), and their expression levels were significantly increased in roots and leaves under P-starvation conditions [44]. PvSPX1 is localized in the nucleus and exhibits a more sensitive and rapid response to P starvation. Overexpression of *PvSPX1* resulted in an increase in P concentration in root tissues, a configuration change of root hairs, growth inhibition of the main root and an increase in the number of lateral roots, accompanied by upregulated transcription of 10 PSR genes [44]. Further studies showed that *PvPHR1* overexpression increased *PvSPX1* transcription level, and thus *PvSPX1* may act downstream from *PvPHR1* [44,50].

4.4. Functional analysis of *SPX* genes in wheat

P deficiency is also a primary factor constraining the yield of wheat [15]. Therefore, identification of P-regulated genes and breeding of low P-tolerant cultivars are of prime importance to increasing global wheat productivity without excessive use of P fertilizers. Owing to the possession of a complex hexaploid genome, functional analysis of P-regulated genes (including *SPX* members) in common wheat ($2n = 6x = 42$) is lagging behind that in *Arabidopsis* and rice. Shang *et al.* [56] found that *TaSPX3* was strongly induced by low P stress, but became significantly downregulated when P supply was restored; the expression profile of *TaSPX3* differed among cultivars, indicating that the mechanism of low P stress response may vary among different wheat genotypes. Shukla *et al.* [57] demonstrated that the relative transcriptional level of *TaSPX1* was higher in the aleurone than in the endosperm in developing wheat grains, which paralleled the accumulation of more P in aleurone tissues.

4.5. Functional analysis of *SPX* genes in rapeseed

Du *et al.* [11] analysed 69 *SPX* gene family members in rapeseed (*Brassica napus*) and found that the expression levels of different *BnaSPX* genes differed under P-starvation conditions. The expression levels of nine genes in the *SPX* subfamilies were significantly induced by P starvation and rapidly declined upon P supplementation. Analysis of two *BnaSPX1* genes (i.e. *BnaA2.SPX1* and *BnaC3.SPX1*) in transgenic *Arabidopsis* revealed functional difference between them: the transgenic lines of *BnaA2.SPX1*, but not those of *BnaC3.SPX1*, showed retarded growth and higher sensitivity to P deficiency when compared with wild-type control [11]. In two other studies, *BnSPX3;1* and *BnSPX3;2* were found

Table 1. List of the plant *SPX* genes whose function has been analysed to some extent. N, cell nucleus; M, cell membrane; C, cytoplasm; +, increase; +(*), increase (except seeds); +(**), increase (except flowers and seeds); =, no difference; −, decrease; Pr, positive regulation; Pr*, positive regulation (except *PvPDR2-like*); Nr, negative regulation.

species	gene	protein location	expression after Pi starvation	regulation of PSI gene	main functional characteristics	source
Oryza sativa	*OsSPX1*	N	+	Nr	OsSPX1 can interact with OsPHR2 and acts as a negative regulator of OsPHR2. OsSPX1 regulates *OsSPX2*, *3* and *5* at the transcriptional level, and the repression of *OsSPX1* results in excessive P accumulation in the shoot	Wang *et al.* [27,28]
	OsSPX2	N	+		OsSPX2 can interact with OsPHR2 and acts as a negative regulator of OsPHR2. PHR2, SPX1 and SPX2 constitute a regulatory feedback loop in P signalling	Wang *et al*. [28,45]
	OsSPX3	N/C	+	Nr	OsSPX3 plays an important role in OsIPS1/miR399-mediated long distance regulation on OsPHO2 and acts as a negative regulator of OsPHR2. OsSPX3 negatively regulates the root-to-shoot transportation of P. Overexpression of *OsSPX3* inhibits plant growth, which is more severe under P-deficient conditions	Wang *et al.* [28] Shi *et al*. [46]
	OsSPX4	N/C	=		OsSPX4 can interact with OsPHR2 in the cytoplasm and inhibits translocation of PHR2 into the nucleus. OsSPX4 functions as a negative regulator of PHR2 and can affect the activity of OsPHR2, sequentially regulating downstream gene expression	Lv *et al*. [47]
	OsSPX5	N/C	+	Nr	*OsSPX5* and *OsSPX3* are paralogous genes. SPX3/5 proteins act as repressors of PHR2. Overexpression of *SPX3* and *SPX5* completely rescues the excessive shoot of P accumulation. SPX3/5 negatively regulates P transport from roots to leaves with redundant function	Shi *et al*. [46] Zhang *et al*. [43]
	OsSPX6		+		*OsSPX6*, as a paralogue of *SPX3/5*, may play a compensatory role	Shi *et al*. [46]

(*Continued*.)

Table 1. (*Continued.*)

species	gene	protein location	expression after Pi starvation	regulation of PSI gene	main functional characteristics	source
Arabidopsis thaliana	*AtSPX1*	N	+	Pr	AtSPX1 can interact with AtPHR1 and may act as a negative regulator of AtPHR1 in P concentration	Duan *et al*. [26] Qi *et al*. [48]
	AtSPX2	N	+		AtSPX2 can interact with AtPHR1 in the cell nucleus. AtSPX1 and AtSPX2 have functional redundancy with one another	Puga *et al*. [49]
	AtSPX3	M/C	+	Nr	Partial repression of *AtSPX3* can exacerbate phosphate-deficiency symptoms, alter P allocation and enhance the expression of a subset of phosphate starvation responsive genes including *AtSPX1*	Duan *et al*. [26]
	AtSPX4	M	−		AtSPX4 can interact with AtPHR1 in the cytoplasm	Duan *et al*. [26]
Glycine max	*GmSPX1*	N/C	+(*)	Nr	GmSPX1 interacts with a newly identified P starvation-induced transcription factor GmMYB48, and this interaction may represent a potential suppressor of P signalling network in soya bean	Zhang *et al*. [43]
	GmSPX2, *4*, *6*, *9* and *10*	N/C	+		—	Yao *et al*. [50]
	GmSPX3, *7* and *8*	N	+		*GmSPX3* overexpression results in increased P concentration in both leaf and root tissues under high P conditions, which correlates with elevated transcript levels of several PSI genes in the root hairs	Yao *et al*. [50]
	GmSPX5	N/C	+(**)		—	Yao *et al*. [50]
Phaseolus vulgaris	*PvSPX1*	N	+	Pr*	Overexpression of *PvSPX1* results in increased P concentration in the roots, morphological change in root hairs, inhibition of main root growth, more numerous lateral roots and upregulated transcription of 10 PSR genes	Yao *et al*. [44]
	PvSPX2	N	+	Pr*	PvSPX2 participates in P signalling pathway in both shoot and root tissues. Overexpression of *PvSPX2* results in increased transcription of several genes downstream from *PvSPX1*, suggesting that PvSPX2 might have a similar regulatory role as PvSPX1	Yao *et al*. [44]
	PvSPX3	N/C	+	=	PvSPX2 participates in P signalling pathway in both shoot and root tissues. *PvSPX3* expression is less sensitive to P deficiency compared with that of *PvSPX1* and *PvSPX2*	Yao *et al*. [44]
Triticum aestivum	*TaSPX129*		+		—	Fang *et al*. [51]
	TaSPX				TaSPX participates in high temperature-induced resistance to wheat stripe rust	Wei *et al*. [52]

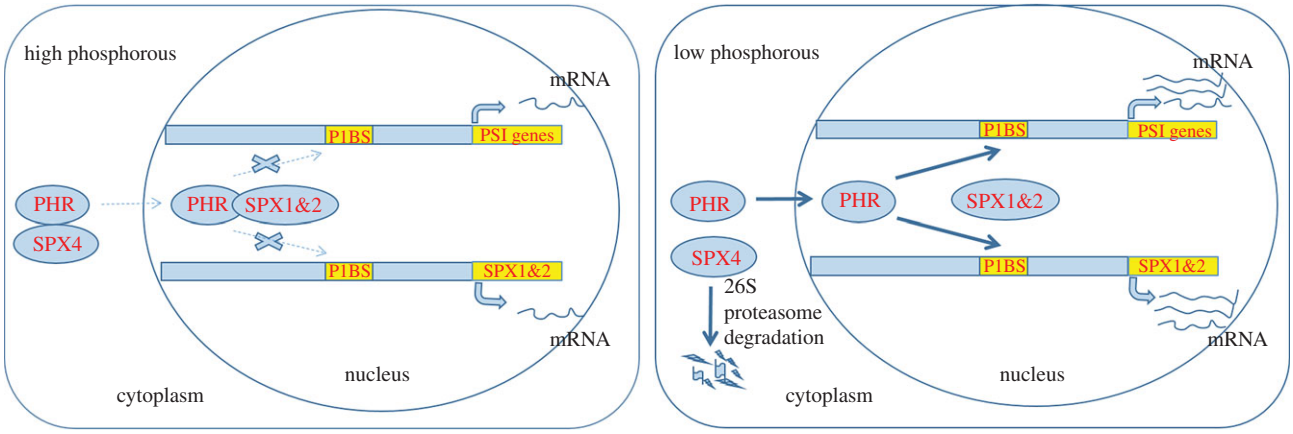

Figure 4. Interaction of SPX1, SPX2 and SPX4 with PHR under high or low P conditions. Under high P conditions, SPX1 and SPX2 in the nucleus and SPX4 in the cytoplasm bind PHR with high affinity, thus inhibiting PHR binding to the P1BS motif in the promoters of PSI genes and leading to repression of the transcription of PSI genes, including *SPX1* and *SPX2*. Under low P conditions, SPX4 in the cytoplasm is degraded via the 26S proteasome pathway, which promotes the targeting of PHR to the nucleus, thereby releasing PHR to activate downstream PSI gene expression. Meanwhile, SPX1 and SPX2 in the nucleus interact with PHR at a low affinity, which facilitates PHR to bind to the P1BS motif in the promoters of PSI genes, further enhancing the expression of PSI genes, including *SPX1* and *SPX2*. The thick arrow denotes enhancement. The dotted lines represent reduced effects. This working model is drawn based on the studies of *SPX1*, *SPX2* and *SPX4* genes in *Arabidopsis* and rice (Lv *et al*. [47]; Wang *et al*. [27,28]; Zhou *et al*. [53]; Puga *et al*. [49]).

specifically induced by P deficiency and that the induction was rapid and reversible [58,59]. Unlike P deficiency, the deprivation of other nutrients (N, K, S or Fe) did not affect the transcription of *BnSPX3;1* and *BnSPX3;2*, and thus the two genes may be used as markers for assessing P-starvation status in plants [58,59].

5. Discussion and prospects

The available studies clearly suggest that SPX proteins occupy a very important position in the P signalling network, which is tightly related to P uptake, transport, storage and homeostasis. Most of the studied *SPX* genes are low P inducible and can influence the transcription of downstream PSI (PSR) genes by regulating PHR activity, likely via controlling the movement of PHR from the cytoplasm to the nucleus and by decreasing the binding of PHR to the P1BS *cis*-element [45,47,49]. Not surprisingly, current understandings on *SPX* genes are largely based on the data from model plants (i.e. *Arabidopsis* and rice). The results from complex crop plants (e.g. legumes, common wheat and rapeseed) are much less. Nevertheless, they complemented and expanded the insights obtained from model species, and yielded potential clues and targets for enhancing crop tolerance to low P stress. Considering the urgent need in developing P-efficient cultivars [60], more efforts should be devoted to studying *SPX* gene functions in crop plants. The accumulation of ever more genomic resources [61], as well as the rapid development of gene-editing technologies for diverse plant species [62], will facilitate such efforts.

Past investigations have mainly concerned relatively simple SPX proteins, i.e. those with only one SPX domain. Future studies should cover more complex SPX proteins that carry extra domains in addition to SPX. The presence of extra domains may confer multiple functions to SPX proteins. This possibility may be illustrated by the analysis of *Arabidopsis* PHO1 (AtPHO1), which carries the EXS domain in addition to SPX. Although originally found required for xylem loading of inorganic phosphate [63,64], recent investigations have indicated the likely involvement of AtPHO1 in the cross talk among P, sucrose and phytohormone signalling pathways [65].

Future studies will also shed new light on the involvement of SPX proteins in other vital plant processes. There is emerging evidence for the participation of SPX domain-containing proteins in disease resistance [52], iron deficiency response [66], low oxygen response [67] and phytochrome-mediated light signalling [68]. Considering the diverse and fundamental roles of P in cellular organisms, it may not be surprising to find that SPX proteins act as important regulatory hubs for multiple processes including the fine tuning of P signalling and homeostasis in plants.

Data accessibility. This article has no additional data.

Author contributions. All authors contributed to the production of this article.

Competing interests. We declare we have no competing interests.

Funding. This study was supported by the Distinguished Scholar Program of Henan Province (154200510024), the Key Project of Henan Province (161100110400) and the Innovation Fund of Henan Agricultural University (KJCX2017A13).

References

1. Wang Y, Ribot C, Rezzonico E, Poirier Y. 2004 Structure and expression profile of the Arabidopsis *PHO1* gene family indicates a broad role in inorganic phosphate homeostasis. *Plant Physiol.* **135**, 400–411. (doi:10.1104/pp.103.037945)
2. Wild R *et al.* 2016 Control of eukaryotic phosphate homeostasis by inositol polyphosphate sensor domains. *Science* **352**, 986–990. (doi:10.1126/science.aad9858)
3. Chen JY. 2008 QTL mapping of phosphorus efficiency and P-related traits in maize (Zea mays

L.). Doctoral dissertation, Southwest University, Chongqing, People's Republic of China. In Chinese.

4. Fang ZY, Shao C, Meng YJ, Wu P, Chen M. 2009 Phosphate signaling in *Arabidopsis* and *Oryza sativa*. *Plant Sci.* **176**, 170–180. (doi:10.1016/j.plantsci.2008.09.007)
5. Secco D, Wang C, Shou H, Whelan J. 2012 Phosphate homeostasis in the yeast *Saccharomyces cerevisiae*, the key role of the SPX domain-containing proteins. *FEBS Lett.* **586**, 289–295. (doi:10.1016/j.febslet.2012.01.036)
6. Marschner H. 1995 *Mineral nutrition of higher plants*, 2nd edn. London, UK: Academic Press.
7. Nussaume L, Kanno S, Javot H, Marin E, Pochon N, Ayadi A, Nakanishi TM, Thibaud MC. 2011 Phosphate import in plants: focus on the PHT1 transporters. *Front. Plant Sci.* **2**, 83. (doi:10.3389/fpls.2011.00083)
8. Liu FP, Zhang LJ, Gu JT, Li XJ, Guo CJ, Lu WJ, Xiao K. 2012 Cloning and molecular characterization analysis of TaPT4, a phosphate transporter gene in wheat (*Triticum aestivum* L.). *J. Agric. Univ. Hebei* **35**, 1–7 (in Chinese). (doi:10.3969/j.issn.1000-1573.2012.03.001)
9. Raghothama KG, Karthikeyan AS. 2005 Phosphate acquisition. *Plant Soil* **274**, 37–49. (doi:10.1007/s11104-004-2005-6)
10. Hinsinger P. 2001 Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* **237**, 173–195. (doi:10.1023/A:1013351617532)
11. Du H, Yang C, Din, G, Shi L, Xu F. 2017 Genome-wide identification and characterization of SPX domain-containing members and their responses to phosphate deficiency in *Brassica napus*. *Front. Plant. Sci.* **8**, 35. (doi:10.3389/fpls.2017.00035)
12. Schachtman DP, Reid RJ, Ayling SM. 1998 Phosphorus uptake by plants: from soil to cell. *Plant Physiol.* **116**, 447–453. (doi:10.1104/pp.116.2.447)
13. Pratt J, Boisson AM, Gout E, Bligny R, Douce R, Aubert S. 2009 Phosphate (Pi) starvation effect on the cytosolic Pi concentration and Pi exchanges across the tonoplast in plant cells: an *in vivo* 31P-nuclear magnetic resonance study using methylphosphonate as a Pi analog. *Plant Physiol.* **151**, 1646–1657. (doi:10.1104/pp.109.144626)
14. Plaxton WC, Tran HT. 2011 Metabolic adaptation of phosphate-starved plants. *Plant Physiol.* **156**, 1006–1015. (doi:10.1104/pp.111.175281)
15. Oono Y, Kobayashi F, Kawahara Y, Yazawa T, Handa H, Itoh T, Matsumoto T. 2013 Characterisation of the wheat (*Triticum aestivum L.*) transcriptome by de novo assembly for the discovery of phosphate starvation-responsive genes: gene expression in Pi-stressed wheat. *BMC Genomics* **14**, 77. (doi:10.1186/1471-2164-14-77)
16. Williamson LC, Ribrioux SP, Fitter AH, Leyser HM. 2001 Phosphate availability regulates root system architecture in Arabidopsis. *Plant Physiol.* **126**, 875–882. (doi:10.1104/pp.126.2.875)
17. Lopez-Bucio J, Hernandez-Abreu E, Sanchez-Calderon L, Nieto-Jacobo MF, Simpson J, Herrera-Estrella L. 2002 Phosphate availability alters architecture and causes changes in hormone sensitivity in the Arabidopsis root system. *Plant Physiol.* **129**, 244–256. (doi:10.1104/pp.010934)
18. Shi T, Zhao D, Li D, Wang N, Meng J, Xu F, Shi L. 2012 *Brassica napus* root mutants insensitive to exogenous cytokinin show phosphorus efficiency. *Plant Soil* **358**, 61–74. (doi:10.1007/s11104-012-1219-2)
19. Vance CP, Uhde-Stone C, Allan DL. 2003 Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytol.* **157**, 423–447. (doi:10.1046/j.1469-8137.2003.00695.x)
20. Gu M, Chen A, Sun S, Xu G. 2016 Complex regulation of plant phosphate transporters and the gap between molecular mechanisms and practical application: What is missing? *Mol. Plant* **9**, 396–416. (doi:10.1016/j.molp.2015.12.012)
21. Luan M, Tang RJ, Tang Y, Tian W, Hou C, Zhao F, Lan W, Luan S. 2016 Transport and homeostasis of potassium and phosphate: limiting factors for sustainable crop production. *J. Exp. Bot.* **68**, 3091–3105. (doi:10.1093/jxb/erw444)
22. Gu M, Chen AQ, Xu GH. 2012 Signaling network in phosphate starvation response and arbuscular mycorrhiza symbiosis in plants. *J. Nanjing Agric. Univ.* **35**, 133–146 (In Chinese).
23. Stefanovic A, Arpat AB, Bligny R, Gout E, Vidoudez C, Bensimon M, Poirier Y. 2011 Over-expression of PHO1 in Arabidopsis leaves reveals its role in mediating phosphate efflux. *Plant J.* **66**, 689–699. (doi:10.1111/j.1365-313X.2011.04532.x)
24. Secco D, Wang C, Arpat BA, Wang Z, Poirier Y, Tyerman SD, Wu P, Shou H, Whelan J. 2012 The emerging importance of the SPX domain-containing proteins in phosphate homeostasis. *New Phytol.* **193**, 842–851. (doi:10.1111/j.1469-8137.2011.04002.x)
25. Chiou TJ, Lin SI. 2011 Signaling network in sensing phosphate availability in plants. *Annu. Rev. Plant Biol.* **62**, 185–206. (doi:10.1146/annurev-arplant-042110-103849)
26. Duan K, Yi K, Dang L, Huang H, Wu W, Wu P. 2008 Characterization of a sub-family of Arabidopsis genes with the SPX domain reveals their diverse functions in plant tolerance to phosphorus starvation. *Plant J.* **54**, 965–975. (doi:10.1111/j.1365-313X.2008.03460.x)
27. Wang C, Ying S, Huang H, Li K, Wu P, Shou H. 2009 Involvement of OsSPX1 in phosphate homeostasis in rice. *Plant J.* **57**, 895–904. (doi:10.1111/j.1365-313X.2008.03734.x)
28. Wang Z, Hu H, Huang H, Duan K, Wu Z, Wu P. 2009 Regulation of OsSPX1 and OsSPX3 on expression of OsSPX domain genes and Pi-starvation signaling in rice. *J. Integr. Plant Biol.* **51**, 663–674. (doi:10.1111/j.1744-7909.2009.00834.x)
29. Jung JY, Ried MK, Hothorn M, Poirier Y. 2017 Control of plant phosphate homeostasis by inositol pyrophosphates and the SPX domain. *Curr. Opin. Biotech.* **49**, 156–162. (doi:10.1016/j.copbio.2017.08.012)
30. Elser JJ. 2012 Phosphorus: a limiting nutrient for humanity. *Curr. Opin. Biotech.* **23**, 833–838. (doi:10.1016/j.copbio.2012.03.001)
31. Nocek B *et al.* 2008 Polyphosphate-dependent synthesis of ATP and ADP by the family-2 polyphosphate kinases in bacteria. *Proc. Natl Acad. Sci. USA* **105**, 17 730–17 735. (doi:10.1073/pnas.0807563105)
32. Livermore TM, Chubb JR, Saiardi A. 2016 Developmental accumulation of inorganic polyphosphate affects germination and energetic metabolism in *Dictyostelium discoideum*. *Proc. Natl Acad. Sci. USA* **113**, 996–1001. (doi:10.1073/pnas.1519440113)
33. Gerasimaite R, Pavlovic I, Capolicchio S, Hofer A, Schmidt A, Jessen HJ, Mayer A. 2017 Inositol pyrophosphate specificity of the SPX-dependent polyphosphate polymerase VTC. *ACS Chem. Biol.* **12**, 648–653. (doi:10.1021/acschembio.7b00026)
34. Hothorn M *et al.* 2009 Catalytic core of a membrane-associated eukaryotic polyphosphate polymerase. *Science* **324**, 513–516. (doi:10.1126/science.1168120)
35. Desfougères Y, Gerasimaite R, Jessen HJ, Mayer A. 2016 Vtc5, a novel subunit of the vacuolar transporter chaperone complex, regulates polyphosphate synthesis and phosphate homeostasis in yeast. *J. Biol. Chem.* **291**, 22 262–22 275. (doi:10.1074/jbc.M116.746784)
36. Müller O, Neumann H, Bayer MJ, Mayer A. 2003 Role of the Vtc proteins in V-ATPase stability and membrane trafficking. *J. Cell Sci.* **116**, 1107–1115. (doi:10.1242/jcs.00328)
37. Wykoff DD, Grossman AR, Weeks DP, Usuda H, Shimogawara K. 1999 Psr1, a nuclear localized protein that regulates phosphorus metabolism in *Chlamydomonas*. *Proc. Natl Acad. Sci. USA* **96**, 15 336–15 341. (doi:10.1073/pnas.96.26.15336)
38. Bari R, Pant BD, Stitt M, Scheible WR. 2006 PHO2, MicroRNA399, and PHR1 define a phosphate-signaling pathway in plants. *Plant Physiol.* **141**, 988–999. (doi:10.1104/pp.106.079707)
39. Martin AC, del Pozo JC, Iglesias J, Rubio V, Solano R, de La Pena A, Leyva A, Paz-Ares J. 2000 Influence of cytokinins on the expression of phosphate starvation responsive genes in Arabidopsis. *Plant J.* **24**, 559–567. (doi:10.1046/j.1365-313x.2000.00893.x)
40. Rubio V, Linhares F, Solano R, Martín AC, Iglesias J, Leyva A, Paz-Ares J. 2001 A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes Dev.* **15**, 2122–2133. (doi:10.1101/gad.204401)
41. Franco-Zorrilla JM, González E, Bustos R, Linhares F, Leyva A, Paz-Ares J. 2004 The transcriptional control of plant responses to phosphate limitation. *J. Exp. Bot.* **55**, 285–293. (doi:10.1093/jxb/erh009)
42. Hammond JP, Broadley MR, White PJ. 2004 Genetic responses to phosphorus deficiency. *Ann. Bot.* **94**, 323–332. (doi:10.1093/aob/mch156)
43. Zhang J, Zhou X, Xu Y, Yao M, Xie F, Gai J, Li Y, Yang S. 2016 Soybean SPX1 is an important

component of the response to phosphate deficiency for phosphorus homeostasis. *Plant Sci.* **248**, 82–91. (doi:10.1016/j.plantsci.2016.04.010)

44. Yao ZF, Liang CY, Zhang Q, Chen ZJ, Xiao BX, Tian J, Liao H. 2014 SPX1 is an important component in the phosphorus signalling network of common bean regulating root growth and phosphorus homeostasis. *J. Exp. Bot.* **65**, 3299–3310. (doi:10.1093/jxb/eru183)
45. Wang Z *et al.* 2014 Rice SPX1 and SPX2 inhibit phosphate starvation responses through interacting with PHR2 in a phosphate-dependent manner. *Proc. Natl Acad. Sci. USA* **111**, 14 953–14 958. (doi:10.1073/pnas.1404680111)
46. Shi J, Hu H, Zhang K, Zhang W, Yu Y, Wu Z, Wu P. 2014 The paralogous SPX3 and SPX5 genes redundantly modulate Pi homeostasis in rice. *J. Exp. Bot.* **65**, 859–870. (doi:10.1093/jxb/ert424)
47. Lv Q *et al.* 2014 SPX4 negatively regulates phosphate signaling and homeostasis through its interaction with PHR2 in rice. *Plant Cell* **26**, 1586–1597. (doi:10.1105/tpc.114.123208)
48. Qi WJ, Manfield IW, Muench SP, Baker A. 2017 AtSPX1 affects the AtPHR1–DNA-binding equilibrium by binding monomeric AtPHR1 in solution. *Biochem. J.* **474**, 3675–3687. (doi: 10.1042/BCJ20170522)
49. Puga MI *et al.* 2014 SPX1 is a phosphate-dependent inhibitor of Phosphate Starvation Response 1 in Arabidopsis. *Proc. Natl Acad. Sci. USA* **111**, 14 947–14 952. (doi:10.1073/pnas.1404654111)
50. Yao Z, Tian J, Liao H. 2014 Comparative characterization of GmSPX members reveals that GmSPX3 is involved in phosphate homeostasis in soybean. *Ann. Bot.* **114**, 477–488. (doi:10.1093/aob/mcu147)
51. Fang WB, Ding WW, Xiao K, Li XJ. 2016 Molecular characterization and expression profile of TaSPX129 in wheat under abiotic stress. *J. Agric. Univ. Hebei.* **39**, 41–45 (In Chinese). (doi:10.13320/j.cnki.jauh.2016.0031)
52. Wei XN, Fan RC, Xu SC, Kang ZS, Zhang XQ. 2011 Function analysis of *Taspx* gene for high temperature resistance to strip rust in wheat cultivar Xiaoyan54. In *Proc. Natl Conf. Plant Biol, Nanning, Guangxi, China,* p. 131 (in Chinese).
53. Zhou Z, Wang Z, Lv Q, Jing S, Zhong Y, Ping W, Mao C. 2015 SPX proteins regulate Pi homeostasis and signaling in different subcellular level. *Plant Signal Behav.* **10**, e1061163. (doi:10.1080/15592324.2015.1061163)
54. Nilsson L, Müller R, Nielsen TH. 2007 Increased expression of the MYB-related transcription factor, PHR1, leads to enhanced phosphate uptake in *Arabidopsis thaliana*. *Plant Cell Environ.* **30**, 1499–1512. (doi:10.1111/j.1365-3040.2007.01734.x)
55. Liu F, Wang Z, Ren H, Shen C, Li Y, Ling HQ, Wu C, Lian X, Wu P. 2010 OsSPX1 suppresses the function of OsPHR2 in the regulation of expression of OsPT2 and phosphate homeostasis in shoots of rice. *Plant J.* **62**, 508–517. (doi:10.1111/j.1365-313X.2010.04170.x)
56. Shang WJ, Jia LH, Shi L, Lin DL, Liu N, Zheng WM. 2016 Screening and expression analysis of genes responded to low phosphate in wheat root. *J. Nanjing Agric. Univ.* **21**, 1–10 (In Chinese).
57. Shukla V, Kaur M, Aggarwal S, Bhati KK, Kaur J, Mantri S, Pandey AK. 2016 Tissue specific transcript profiling of wheat phosphate transporter genes and its association with phosphate allocation in grains. *Sci. Rep.* **6**, 39293. (doi:10.1038/srep39293)
58. Yang G, Ding G, Shi L, Cai H, Xu F. 2012 Characterization of phosphorus starvation-induced gene BnSPX3 in *Brassica napus*. *Plant Soil* **350**, 339–351. (doi:10.1007/s11104-011-0913-9)
59. Yang GZ. 2011 Isolation and identification of phosphorus starvation-induced gene BnSPX3, BnIPS1 and their promoters in Brassica napus. Doctoral dissertation, Huazhong Agric University, Wuhan, People's Republic of China (In Chinese).
60. Möller K. 2013 Improving the phosphorus efficiency of organic farming systems. Core Organic Newsletter.
61. Varshney RK, Glaszmann JC, Leung H, Ribaut JM. 2010 More genomic resources for less-studied crops. *Trends Biotechnol.* **28**, 452–460. (doi:10.1016/j.tibtech.2010.06.007)
62. Gupta RM, Musunuru K. 2014 Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. *J. Clin. Invest.* **124**, 4154–4161. (doi:10.1172/JCI72992)
63. Hamburger D, Rezzonico E, MacDonald-Comber Petétot J, Somerville C, Poirier Y. 2002 Identification and characterization of the Arabidopsis *PHO1* gene involved in phosphate loading to the xylem. *Plant Cell* **14**, 889–902. (doi:10.1105/tpc.000745)
64. Poirier Y, Thoma S, Somerville C, Schiefelbein J. 1991 A mutant of Arabidopsis deficient in xylem loading of phosphate. *Plant Physiol.* **97**, 1087–1093. (doi:10.1104/pp.97.3.1087)
65. Ribot C, Wang Y, Poirier Y. 2008 Expression analyses of three members of the AtPHO1 family reveal differential interactions between signaling pathways involved in phosphate deficiency and the responses to auxin, cytokinin, and abscisic acid. *Planta* **227**, 1025–1036. (doi:10.1007/s00425-007-0677-x)
66. Nakanishi H, Okumura N, Umehara Y, Nishizawa NK, Chino M, Mori S. 1993 Expression of a gene specific for iron deficiency (Ids3) in the roots of *Hordeum vulgare*. *Plant Cell Physiol.* **34**, 401–410. (doi:org/10.1093/oxfordjournals.pcp.a078434)
67. Sell S, Hehl R. 2005 A fifth member of the tomato 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase gene family harbours a leucine zipper and is anaerobically induced. *DNA Seq.* **16**, 80–82. (doi:10.1080/10425170500050817)
68. Kang X, Ni M. 2006 Arabidopsis SHORT HYPOCOTYL UNDER BLUE1 contains SPX and EXS domains and acts in cryptochrome signaling. *Plant Cell* **18**, 921–934. (doi:10.1105/tpc.105.037879)