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Transcription factors PHR1 and PHR1-like 1 regulate ABA-mediated inhibition of seed germination and stomatal opening in *Arabidopsis*

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ABSTRACT

Low phosphate (LP) availability significantly impacts crop yield and quality. PHOSPHATE STARVATION RESPONSE1 (PHR1) along with PHR1-like 1 (PHL1) act as a key transcriptional regulator in a plant's adaptive response to LP conditions. Abscisic acid (ABA) plays an important role in how plants respond to environmental stresses like salinity and drought. However, the involvement of PHR1 and PHL1 in ABA response and signalling mechanisms remains to be fully understood. Our findings reveal that *PHR1* and *PHR1/PHL1* knockout mutations enhance the responsiveness of seed germination, early seedling growth, and stomatal opening to ABA in *Arabidopsis*. Furthermore, these mutations increase sensitivity to combined LP and ABA stress. In contrast, overexpression of *PHR1* or *PHL1* reduces this sensitivity in *Arabidopsis*. Knockout mutations of *PHR1* and *PHR1/PHL1* also increase sensitivity to salt and osmotic stresses, as well as to combined LP and salinity/osmotic stress, while overexpression of *PHR1* or *PHL1* reduces their sensitivity in seed germination and early seedling development. Knockout mutations of *SPX1* and *SPX2*, negative regulators of PHR1 and PHL1, decrease sensitivity to ABA and salt/osmotic stresses in *Arabidopsis*. A group of genes related to ABA metabolism and signalling is significantly affected by the knockout or overexpression of *PHR1* and *PHL1*, with a large proportion of these genes containing PHR1 binding site (P1BS) in their promoters. Moreover, the ABA-sensitive phenotype of *phr1* or *phr1 phl1* mutants can be rescued by PHR1 homologs from chlorophyte algae, liverwort and rice, suggesting their conserved roles in ABA signalling. These results indicate that PHR1 and its homologs negatively regulate plant responses to ABA in seed germination and stomatal aperture. This study provides new insights into the interplay between Pi homeostasis, abiotic stress and ABA signaling. Moderately increasing the expression of *PHR1* or its homologs in crops could be a potential strategy to enhance plant resistance to combined LP and osmotic stress.

1. Introduction

Phytohormone ABA plays pivotal roles in stress response and tolerance in plants by regulating various physiological activities including maintaining seed dormancy, inhibiting seed germination, and modulating stomatal aperture (Finkelstein, 2013; Hsu et al., 2021; Nakashima and Yamaguchi-Shinozaki, 2013). Under drought and osmotic stresses, ABA is released in plants to decrease stomatal apertures and reduce water loss (Hsu et al., 2021). During seed development, ABA concentration is increased to promote the maturation and dormancy of seeds (Finkelstein, 2013; Ali et al., 2022). The core pathway for ABA signaling is now well understood. ABA binds to its receptors-PYR/PYL/RCARs, which then interact with type 2 C protein phosphatases (PP2Cs). This interaction inhibits PP2Cs, preventing the dephosphorylation of SNF1-RELATED PROTEIN KINASE 2 s (SnRK2s) and thereby sustaining SnRK2 activity. The released SnRK2s subsequently phosphorylate downstream target proteins including ion transporters and transcription factors, thus altering turgor in guard cells and mediating seed maturation and dormancy (Finkelstein, 2013; Fujii et al., 2011; Ali et al., 2022; Chen et al., 2020).

Transcription factors play a crucial role in the molecular regulatory network of ABA signaling. Notably, members of the ABA-responsive element (ABRE)-binding protein/ABRE-binding factor (AREB/ABF) family, such as ABF1, ABF2, ABF3, and ABF4 are significant contributors

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to this pathway (Fujita et al., 2013). In response to environmental stresses and developmental cues, AREBs/ABFs become phosphorylated and activated by SnRK2s. This activation leads to the induction of ABA-responsive genes, including *RD29A* and *RD29B* (Fujita et al., 2013; Wang et al., 2019; Zhao et al., 2020). The transcription factors ABSCISIC ACID INSENSITIVE 3 (ABI3), ABI4 and ABI5, which belong to basic B3, AP2/ERF and bZIP families, respectively, work together to mediate ABA signaling (Finkelstein, 2013; Lopez-Molina et al., 2001; Nakamura et al., 2001). The expression of NAC (NAM, ATAF, and CUC) transcription factors including *RD26*, is induced by ABA and osmotic stress. These transcription factors play a crucial role in regulating ABA signaling, as well as in the plant's response to stress and its tolerance to adverse conditions (Fujita et al., 2004). *Arabidopsis* NAC072 and ANAC096 can interact with ABF3 and ABF2/4, respectively, to mediate the expression of ABA- and/or drought-responsive genes (Li et al., 2016; Xu et al., 2013). AP2/ERF transcription factors (such as DREB1A, DREB2A, and DERB2C) also regulate the expression of ABA-related genes and stress response through interactions with ABF2/3/4 (Lee et al., 2010). Constitutive expression of transcription factors like *WRKY57*, *CAMTA3* and *IDD14* confers drought tolerance by activating ABA biosynthesis and/or signaling (Jiang et al., 2012; Liu et al., 2022; Zeng et al., 2022).

Phosphorus (P) is an important macronutrient essential for the growth and productivity of plants. However, a considerable amount of P in the soil becomes unavailable for plant uptakes due to mechanisms such as adsorption to soil particles, precipitation into insoluble compounds, or conversion to organic forms. Therefore, plants frequently suffer inorganic phosphate (Pi) limitations (Raghothama, 1999). Under low Pi (LP) conditions, plants adopt many strategies to maintain cellular Pi homeostasis. These include enhancing Pi acquisition by roots, remobilizing Pi from old leaves to young tissues, and coordinating Pi translocation and allocation (Madison et al., 2023; Vance et al., 2003). Through genetic and molecular approaches, many genes have been demonstrated to participate in the regulation of Pi homeostasis and LP responses (Gu et al., 2016; Lopez-Arredondo et al., 2014; Lu et al., 2023; Yang et al., 2024). Among them, MYB-coiled-coil (MYB-CC) transcription factors, including *PHOSPHATE STARVATION RESPONSE1* (*PHR1*) and its closely related homologs like *PHR1-like 1* (*PHL1*), play pivotal roles in controlling the transcription of various phosphate starvation responsive (PSR) genes. Notable examples include *ACID PHOSPHATASE5* (*ACP5*), miRNA399, and the phosphate transporters *PHOSPHATE TRANSPORTER1;1* (*Pht1;1*) and *Pht1;4* (Yang et al., 2024). *Arabidopsis* PHR1 and PHL1 bind to a *cis*-element called PHR1-binding site (P1BS, GNATATNC) in promoters of many PSR genes (Rubio et al., 2001; Bustos et al., 2010). Although the mutation of *PHL1* has little effect on PSR gene expression, the expression of some PSR genes is further reduced in the double mutant *phr1 phl1* when compared with the single mutant *phr1*, suggesting some redundancy in PHR1 and PHL1 functions (Bustos et al., 2010). Recently, PHR1/PHL1 and PHL2/PHL3 have been shown to function as two distinct modules in the regulation of LP responses (Wang et al., 2023). SPX (SYG1, Pho81 and XPR1) proteins function as Pi sensors by binding with inositol polyphosphates (InsPs), which reflect cellular Pi levels (Jia et al., 2021; Wang et al., 2021). Under conditions with sufficient Pi, the inositol pyrophosphate InsP8 is accumulated and the binding of $InsP_8$-SPX complex to the CC domain of PHRs restrains PHRs-mediated transcriptional regulation of PSR genes (Dong et al., 2019; Zhou et al., 2021; Zhu et al., 2019). By contrast, under Pi starvation, Pi, ATP and InsP8 drop, SPX-PHR complex dissociates, and PHR1/PHL1 promotes PSR gene expression (Riemer et al., 2021). It is notable that the formation of SPX-PHR complexes and their roles in the regulation of Pi starvation responses is highly conserved in plants including chlorophyte algae and non-vascular bryophyte (Jia et al., 2023; Nezamivand-Chegini et al., 2021; Zhang et al., 2016). Similar to PHR1/PHL1 in *Arabidopsis*, its orthologs in other plant species, such as *Chlamydomonas reinhardtii CsPSR1* (Bajhaiya et al., 2016), liverwort *MpPHR1*) (Rico-Resendiz et al., 2020), and rice *OsPHR2* (Zhou et al., 2008), also play central roles in plant responses to LP. Under LP stress, SPX proteins are degraded through protein ubiquitination, resulting in the release of PHRs and the expression of downstream PSR genes (Ruan et al., 2019). Additionally, three other SPX-related gene subfamilies including SPX-EXS, SPX-RING and SPX-MFS, which are named based on additional domains in their structures, are also involved in Pi homeostasis. For example, SPX-EXS protein PHO1 is involved in root-to-shoot translocation of Pi (Dai et al., 2024; Rouached et al., 2011); SPX-MFS protein VPT1 (Vacuolar Phosphate Transporter 1, also called PHT5;1) is involved in the sequestration of vacuolar Pi, and is important for plant adaptation to the varying external Pi availability (Liu et al., 2015, 2016); SPX-RING protein NLA functions as an E3 ligase targeting Pi transporter PHT1 as well as PHR1 for 26S proteasome-mediated degradation (Lin et al., 2013; Park et al., 2023). In addition, the interaction between Pi nutrition and ABA signaling is partially explored. The level of ABA was found to increase under Pi deficiency in plants like *Arabidopsis*, soybean and castor bean (Jaschke et al., 1997; Zhang et al., 2022; Castro-Valdecantos et al., 2023). Lots of ABA- and abiotic stress-responsive genes were found to be affected by Pi deprivation (Scheible et al., 2023; Woo et al., 2012). ABA-mediated stomatal closure is known to crosstalk with Pi starvation response through PHO1 in *Arabidopsis* (Zimmerli et al., 2012). ABI5, a key component in ABA signaling, has been suggested to regulate Pi uptake by regulating the expression of PSR genes like *PHT1;5* (Lei et al., 2022; Zhang et al., 2022). But how Pi signaling components like PHR1/PHL1 as well as their homologs interplay with ABA response and signaling is still unclear.

PHR1 and PHL1 are core transcriptional regulators controlling PSR gene expression in the physiological and metabolic responses to Pi deficiency (Nilsson et al., 2007; Pant et al., 2015; Rubio et al., 2001; Bustos et al., 2010; Sun et al., 2016). It has been shown that PHR1 is involved in multiple stress responses and resistance, including high light stress adaptation under LP conditions (Nilsson et al., 2012); stress-related proline metabolism (Aleksza et al., 2017); plant response to illuminated hypoxia stress (Klecker et al., 2014); biotrophic pathogen defenses (Scheible et al., 2023; Castrillo et al., 2017). In this study, we show PHR1 and PHL1 are involved in regulating ABA-mediated inhibition of seed germination and stomatal opening, and are involved in regulating seed germination and early seedling development under combined LP and salt as well as combined LP and osmotic stresses.

2. Materials and methods

2.1. Plant material and growth conditions

This study utilized *Arabidopsis thaliana* plants of the Colubia-0 (Col-0) ecotypes. The mutants analyzed were *phr1* (SALK_067629), *phl1* (SALK_079505), and *spx1 spx2* double mutant (SALK_092030; SALK_080503), previously established and characterized in related research (Dai et al., 2024). PHR1-complemented lines (*ProPHR1:PHR1/phr1*; ProPHR1:CrPSR1/*phr1phl1*; ProPHR1:MpPHR1/*phr1phl1*; ProPHR1:OsPHR2/*phr1*), *PHR1*-overexpression lines (*PHR1-OE*), and *PHL1*-overexpression lines (*PHL1-OE*) were constructed in this study. The genotyping and expression levels of these mutants or transgenic lines were checked. Seeds were sterilized by immersing them in 75 % ethanol for 10 minutes, then rinsed in sterile water and stratified at 4 ℃ for two days. After stratification, seeds were sown on 1/2 Murashige and Skoog (MS) medium (pH 5.7) supplemented with 1 % agar and 1 % sucrose. After seven days' growth, seedlings were transplanted from the MS medium to soils and cultivated in a growth chamber set to a 16-hour light/8-hour dark cycle, with a day/night temperature of 20℃/ 22 ℃. Hydroponic culture of *Arabidopsis* was performed according to a previous study (Zeng et al., 2018).

2.2. Seed germination assay

Seeds were sterilized by immersing them in 75 % ethanol for

10 minutes, followed by two washes with distilled water. They were then sown on 1/2 MS media with either standard or low phosphate (10 μM) concentrations, and supplemented with ABA, NaCl, or mannitol according to the experimental design. After sowing, the plates were stored at 4 ℃ in darkness for two days to promote stratification. Subsequently, they were transferred to a growth chamber with a 16-hour light/8-hour dark cycle at 20℃/22 ℃ for germination analyses. Each condition was tested with a minimum of 40 seeds across three biological replicates. Germination was marked by the visible emergence of the radicle, and the cotyledon greening rate was recorded by counting the seedlings with fully green cotyledons at specific intervals.

2.3. Vector construction and plant transformation

For the construction of *Arabidopsis PHR1/PHL1* overexpression (35S: PHR1/PHL1), the coding sequence (CDS) of *PHR1/PHL1* without a stop codon was cloned into the plant expression vector pCAMBIA1300 harboring a C-terminal 3FLAG tag, which was driven by 35S promoter, by using the method of homologous recombination. For the construction of complementation, the *PHR1* promoter region (1.6 kb) was amplified from wild-type (WT) genomic DNA and cloned into pCAMBIA1300 expression vector to replace 35S promoter, then the CDS of *AtPHR1* (At4g28610), *CrPSR1* (Cre12.g495100), *MpPHR1* (Mapoly0003s0147), and *OsPHR2* (LOC_Os07g25710), were amplified by using complementary DNA (cDNA) as a template and inserted after *PHR1* promoter by the method of homologous recombination. The recombinant plasmid was introduced into *Agrobacterium tumefactions* strain GV1301 and subsequently utilized to transform WT plants, along with *phr1* or *phr1 phl1* mutant lines for complementation studies, employing the floral dip technique (Clough and Bent, 1998). Homozygous lines with AtPHR1/AtPHL1 overexpression, as well as AtPHR1/CrPSR1/MpPHR1/OsPHR2 complementation, were used for seed germination analyses.

2.4. Measurement of Pi concentration

The Pi content in plant tissues was quantified using a method adapted from a previous study (Zeng et al., 2018). Fresh plant tissue (about 0.5 g) was finely ground under liquid nitrogen, and suspended in an extraction buffer composed of 1 mM EDTA, 10 mM Tris, 100 mM NaCl, and 1 mM β-mercaptoethanol, pH 8.0. After homogenization, 1 % glacial acetic acid was added, and the mixture was incubated at 42 ℃ for 30 minutes. The samples were then centrifuged at 10,000 g for 10 minutes at 4 ℃. A 1.0 mL aliquot of the resulting supernatant was mixed with 2.0 mL working solution reagent [0.34 % ammonium molybate ($(NH_4)_6Mo_7O_{24}{\cdot}4\ H_2O$), 1.4 % ascorbic acid, and 0.46 M sulfuric acid (H_2SO_4)]. This mixture was incubated at 40 ℃ for 20 minutes, cooled to room temperature, and then measured at the absorbance of 820 nm by using a UV-Vis spectrophotometer (Thermo Scientific BioMate 3S). Pi concentrations were determined by comparing the absorbance values to a standard phosphate calibration curve.

2.5. Stomatal aperture assay

Stomatal apertures were measured using a refined method based on previous study (Ding et al., 2023). Leaves from 4-week-old plants grown with hydroponic culture were blended in a Waring blender with deionized water to create homogenate. The epidermal fragments were then filtered through a nylon mesh to isolate the epidermal peels. These peels were incubated in the stomatal opening buffer containing 5 mM MES-bistrispropane, 0.1 mM $CaCl_2$, and 50 mM KCl, adjusted to pH 6.5, for 3 hours at 22 ℃ under light intensity of approximately 250 μmol m^{-2} s^{-1}, ensuring the complete opening of the stomatal guard cells. Subsequently, ABA was introduced to the buffer at a final concentration of 10 μM, and stomatal apertures were assessed after a 3-hour incubation. The stomatal opening was captured using a digital camera connected to a light microscope (Nikon). For each sample replicate, at least 60 stomata were examined, and the widths of the stomatal apertures were quantified using ImageJ software (Abramoff et al., 2004).

2.6. Water loss assay of leaves

Three to four rosette leaves of similar age were detached from 3 to 4 week-old plants grown with hydroponic culture, and their weight was measured at room temperature with a relative humidity of 40 %-60 %. The initial biomass of the leaves at 0 hours was set to 100 %, and their weight was recorded every hour for a total of 6 hours. Each sample had five replicates.

2.7. Transcriptome datasets, gene ontology (GO) enrichment, and promoter analysis

Transcriptome datasets for differentially expressed genes (DEGs) in responses to Pi deficiency, as well as DEGs in *phr1* and *phr1 phl1* mutants, were retrieved from published microarray data (Bustos et al., 2010). GO term enrichment analysis of DEGs in *phr1* and *phr1 phl1* mutants was performed using the online GO enrichment analysis tool (http://plantregmap.gao-lab.org/go.php) with significantly enriched GO terms identified based on an adjusted P-value $\leq$ 0.05. Promoter sequences, spanning 2.0 kb upstream to the transcription start site, were retrieved from the TAIR database (http://www.arabidopsis.org/), and the location of the P1BS *cis*-acting regulatory element was analyzed using the online Regulatory Sequence Analysis Tools (http://www.rsat.eu/).

2.8. Quantitative RT-PCR analysis

Total RNA was isolated using the Ultrapure RNA Kit (CWBIO, Jiangsu, China). The synthesis of cDNA was carried out with the HiScript®III All-in-one RT SuperMix Perfect for qPCR (Vazyme, Nanjing, China). For real-time quantitative PCR, Taq Pro Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China) was employed. Gene expression levels were normalized to that of internal reference genes *ACTIN2* (At3g18780) and *TUBULIN2* (At5g62690), and results were expressed as $2^{-\Delta\Delta CT}$. The sequences of primers specific to the target genes are provided in Table S1.

3. Results

3.1. PHR1 and PHL1 knockouts increase ABA and LP sensitivity in seed germination

Using the online eFP tool to analyze gene expression patterns (Waese et al., 2017), it was found that *PHR1* and *PHL1* are expressed across various tissues during *Arabidopsis* growth and development (Fig. S1). The expression level of *PHR1* was higher than that of *PHL1* in diverse tissues, including mature leaves, flowers, and seeds during germination (Fig. S2). Analysis of abiotic-stress-microarray data revealed that *PHR1* was slightly repressed by osmotic and heat stresses, while *PHL1* was induced by osmotic and salt stresses (Fig. S3). Additionally, microarray data indicated that *PHR1* and *PHL1* were expressed in guard cells, and *PHL1* was slightly repressed by ABA in guard cells but induced by ABA in mesophyll cells (Fig. S4). These results suggest that *PHR1* and *PHL1* may play roles in plant responses to ABA and/or osmotic stress.

Using T-DNA insertion mutants, including *phr1*, *phl1*, and *phr1 phl1*, we tested the role of *PHR1* and *PHL1* in ABA signaling. The disruption of expression due to T-DNA insertion was confirmed (Fig. S5A), and the shoot Pi concentration was significantly reduced in the knockout mutants of *PHR1* and *PHL1* in *phr1* and *phr1 phl1* (Fig. S5B), consistent with previous findings (Bustos et al., 2010). Under control conditions, the seed germination rates and cotyledon greening rates in *phr1*, *phl1*, and *phr1 phl1* mutants showed no obvious differences compared with Col-0

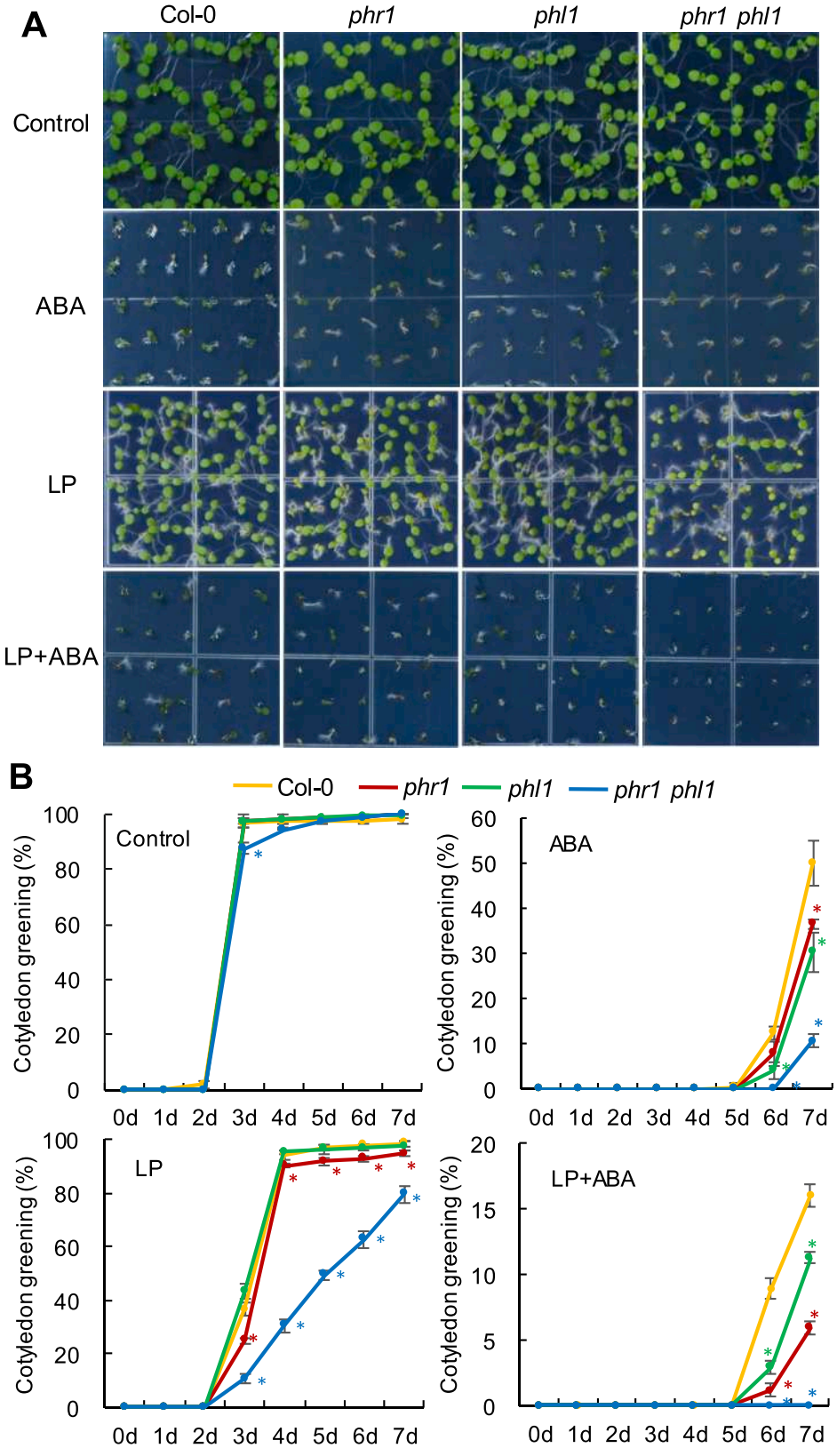

Fig. 1. Seed germination of *phr1*, *phl1* and *phr1 phl1* mutants in responses to ABA, LP, and combined LP+ABA treatments. (A) Phenotypes of Col-0, *phr1*, *phl1* and *phr1 phl1* seed germination under treatments of ABA, LP and combined LP+ABA for 7 days. (B) Cotyledon greening rates of *phr1*, *phl1* and *phr1 phl1* mutant seeds under treatments of ABA, LP, and combined LP+ABA. Seeds were germinated and grown under different treatments for seven days (ABA: 1/2 MS with 0.5 μM ABA; LP: 1/2 MS with 10 μM Pi; LP+ABA: 1/2 MS with 10 μM Pi and 0.5 μM ABA). Values are means ± SD of three replicates (at least 40 seeds were used for each replicate). Asterisk indicates a significant difference when compared with that of Col-0 by using Student's *t*-test (*P < 0.05).

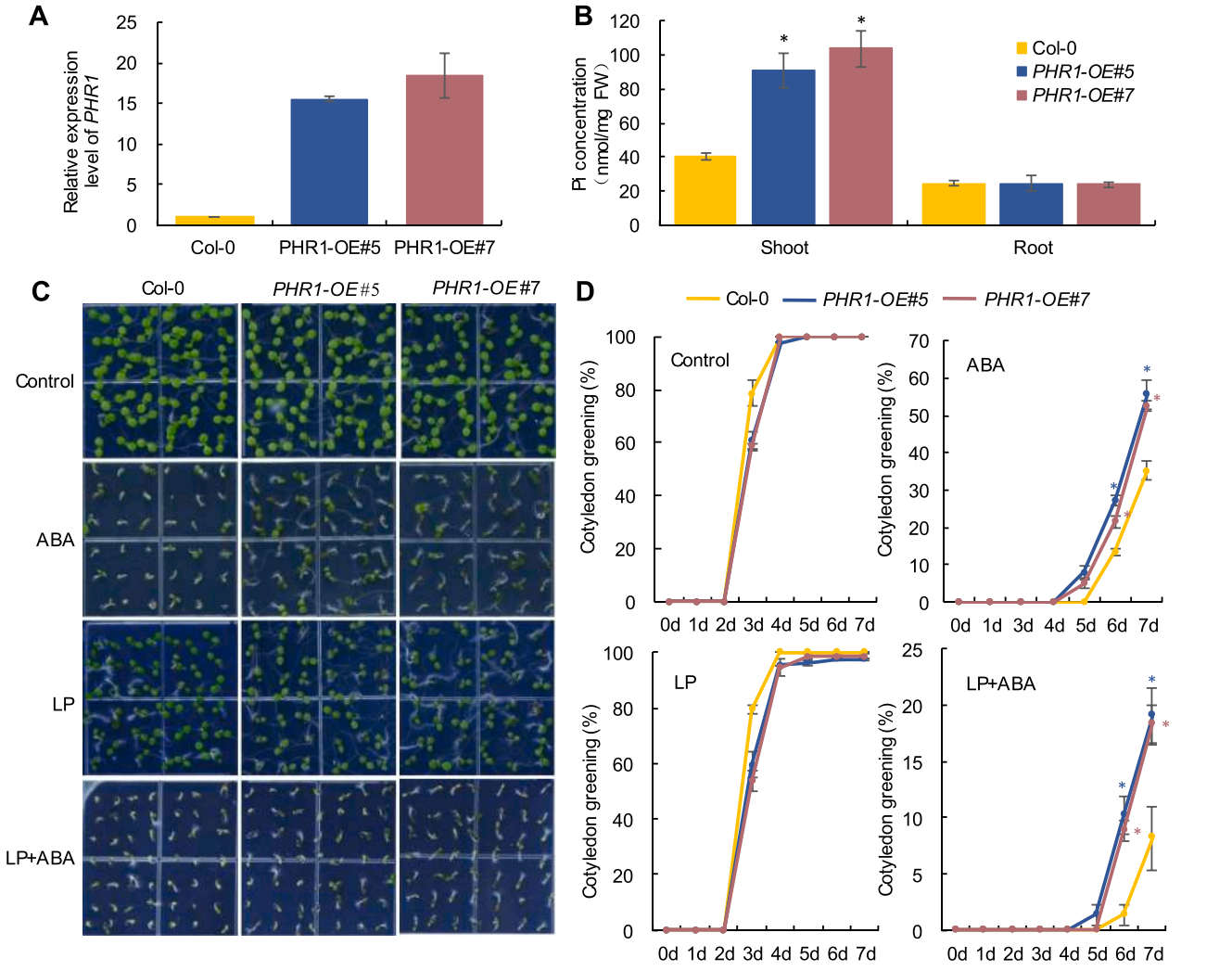

Fig. 2. Seed germination of *PHR1*-overexpression plants in responses to ABA, LP, and combined LP+ABA treatments. (A) Relative expression level of *PHR1* in *PHR1*-overexpression seedlings (PHR1-OE#5 and PHR1-OE#7). (B) Pi concentration of *PHR1*-overexpression plants in shoots and roots. (C) Seed germination phenotypes of *PHR1*-overexpression lines under treatments of ABA, LP and combined LP+ABA. (D) Cotyledon greening rates of *PHR1*-overexpression seeds under treatments of ABA, LP, and combined LP+ABA. Seeds were germinated and grown under different treatments for seven days (ABA: 1/2 MS with 0.5 μM ABA; LP: 1/2 MS with 10 μM Pi; LP+ABA: 1/2 MS with 10 μM Pi and 0.5 μM ABA). Values are means ± SD of three replicates (at least 40 seeds were used for each replicate). Asterisk indicates a significant difference when compared with that of Col-0 by using Student's *t*-test (*P < 0.05).

(WT) (Fig. 1; Fig. S6). At day 5 of ABA treatment, the seed germination of *phr1*, *phl1*, and *phr1 phl1* was slightly lower than that of WT (Fig. S6). At day 6 and 7 of ABA treatment, the cotyledon greening rates were significantly lower in the mutants compared to WT, with *phr1 phl1* showing an ever greater reduction than the single mutants of *phr1* or *phl1* (Fig. 1). These results suggested PHR1 and PHL1 negatively regulate ABA-mediated inhibition of seed germination and early seedling development.

Since PHR1 and PHL1 are central transcription factors in LP stress response, we analyzed the seed germination in these mutants under LP and combined LP and ABA (LP+ABA) conditions. No significant differences in seed germination rates were observed between the mutants and WT under LP alone (Fig. S6). Interestingly, the cotyledon greening rate under LP was reduced in the *phr1* mutant, but not in the *phl1*, and was dramatically suppressed in the *phr1 phl1* double mutant compared to single mutants (Fig. 1). The combined LP+ABA treatment further exacerbated the reduced seed germination rates and cotyledon greening rates of *phr1* and *phr1 phl1* mutants (Fig. 1). These results suggest that PHR1 and PHL1 play overlapping roles in LP- and ABA-mediated inhibition of seed germination and early seedling development, with additive effects of LP and ABA on the repression of cotyledon greening.

Complementation lines of *phr1* (pPHR1:PHR1/*phr1*#7, #9) were generated and tested for their responses to ABA and LP. *PHR1* expression was restored to WT levels, and the cotyledon greening rate was rescued to the level of WT in the complementation lines under ABA, LP, and LP+ABA treatments (Fig. S7). These results confirm that the delayed seed germination and cotyledon greening in *phr1* under ABA and LP treatments is due to the loss of function mutation of *PHR1*.

3.2. Overexpression of PHR1 reduces the sensitivity of seed germination to combined ABA and LP

The overexpression of *PHR1* lines was used to further investigate the role of PHR1 in LP- and ABA-mediated inhibition of seed germination. The expression levels of *PHR1* were significantly higher in the overexpression lines compared to WT (Fig. 2A). The shoot Pi concentration in the *PHR1*-overexpression lines was significantly increased compared to WT, although no significant change was observed in root Pi concentration (Fig. 2B). Under ABA treatments, the seed germination and cotyledon greening rate in the *PHR1*-overexpression lines were

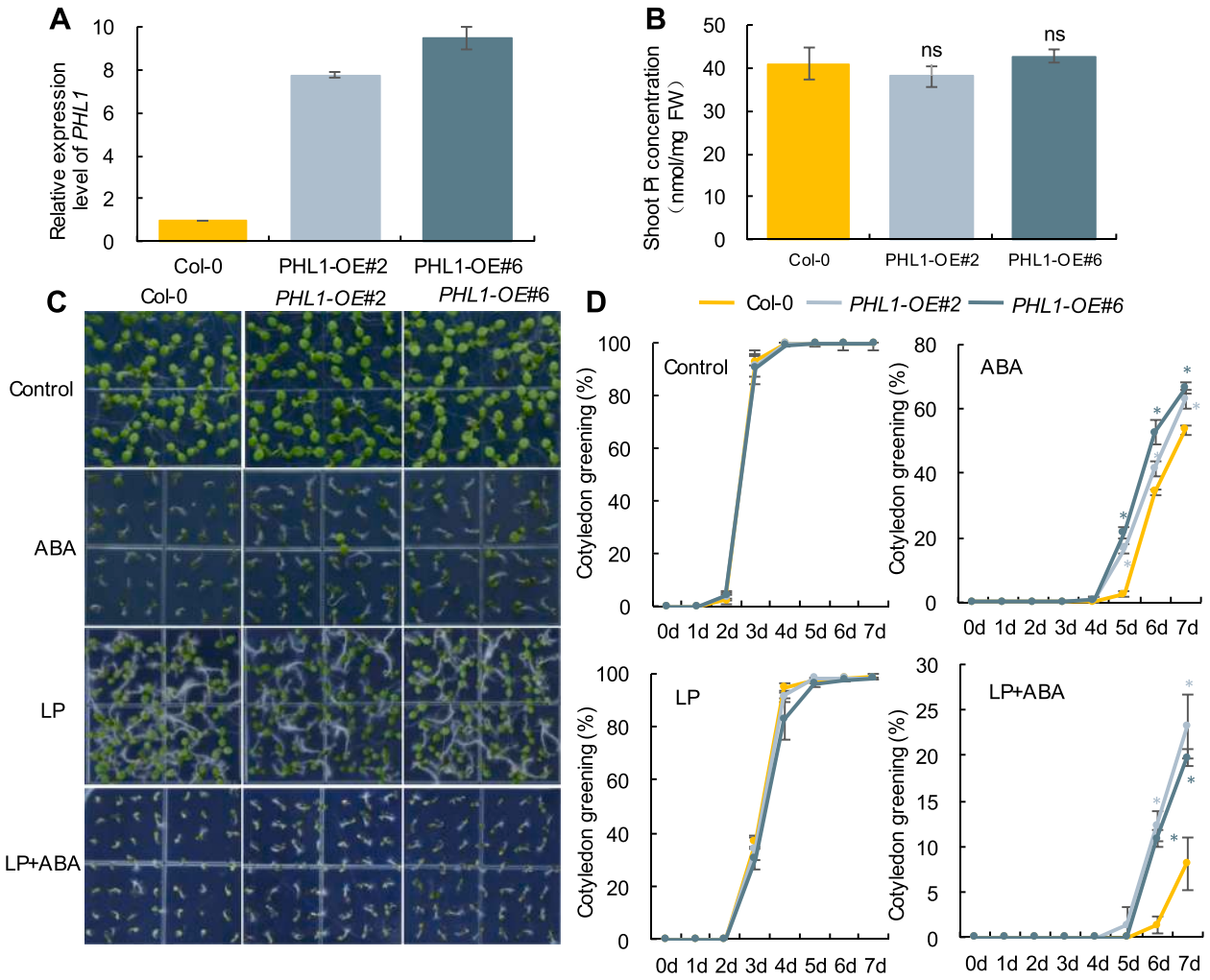

Fig. 3. Seed germination of *PHL1*-overexpression plants in responses to ABA, LP, and combined LP+ABA treatments. (A) Relative expression level of *PHL1* in *PHL1*-overexpression seedlings (*PHL1-OE#2* and *PHL1-OE#6*). (B) Shoot Pi concentration of *PHL1*-overexpression plants. (C) Seed germination phenotypes of *PHL1*-overexpression lines under treatments of ABA, LP and combined LP+ABA. (D) Cotyledon greening rates of *PHL1*-overexpression seeds under treatments of ABA, LP, and combined LP+ABA. Seeds were germinated and grown under different treatments for seven days (ABA: 1/2 MS with 0.5 μM ABA; LP: 1/2 MS with 10 μM Pi; LP+ABA: 1/2 MS with 10 μM Pi and 0.5 μM ABA). Values are means ± SD of three replicates (at least 40 seeds were used for each replicate). Asterisk indicates a significant difference when compared with that of Col-0 by using Student's *t*-test (*P < 0.05).

remarkably higher than those of WT (Fig. 2C-D; Fig. S8). While there was no significant difference in seed germination between the overexpression lines and WT under LP alone, the difference in seed germination increased under LP+ABA compared to LP alone (Fig. 2D; Fig. S8). These results suggest that PHR1 negatively regulates LP- and ABA-mediate inhibition of seed germination in *Arabidopsis*.

3.3. Overexpression of PHL1 reduces the sensitivity of seed germination to combined ABA and LP

The *PHL1*-overexpression lines were used to investigate the role of *PHL1* in ABA- and LP-mediated inhibition of seed germination. The expression levels of *PHL1* were significantly elevated in the overexpression lines (Fig. 2A). No significant changes in root or shoot Pi concentrations were observed in the *PHL1*-overexpression lines compared to WT (Fig. 2B). The seed germination and cotyledon greening rates of *PHL1*-overexpression lines were significantly higher than those of WT under ABA treatment, but no significant difference was observed under LP treatment (Fig. 2C-D; Fig. S9). Under LP+ABA treatment, the seed germination rates were further improved by *PHL1* overexpression; with cotyledon greening rates being twice as high as WT after 7 days of treatment (Fig. 3D). These results suggest that PHL1, like PHR1, negatively regulates LP- and ABA-mediate inhibition of seed germination in *Arabidopsis*.

3.4. Knockout mutations of SPX1 and SPX2 increase seed germination under combined ABA and LP treatment

Since SPX1 and SPX2 function in Pi signaling by repressing PHR1 activity through protein interactions in the nucleus, sequestering PHR1 from binding to the P1BS element (Puga et al., 2014), we used *spx1 spx2* double mutant to investigate the effect of knockout mutation of *SPX1* and *SPX2* on ABA signaling. The shoot Pi concentration in the *spx1 spx2* double mutant was significantly higher, while the root Pi concentration was lower compared to WT (Fig. S10A). Consistent with our hypothesis, the seed germination and cotyledon greening phenotypes of *spx1 spx2* double mutant resembled those of the *PHR1*- or *PHL1*-overexpression lines, showing significantly higher rates than WT under ABA and LP+ABA treatments (Fig. 4; Fig. S11). These results suggested that SPX1 and SPX2 are involved in ABA signaling by repressing the activities of PHR1 and PHL1.

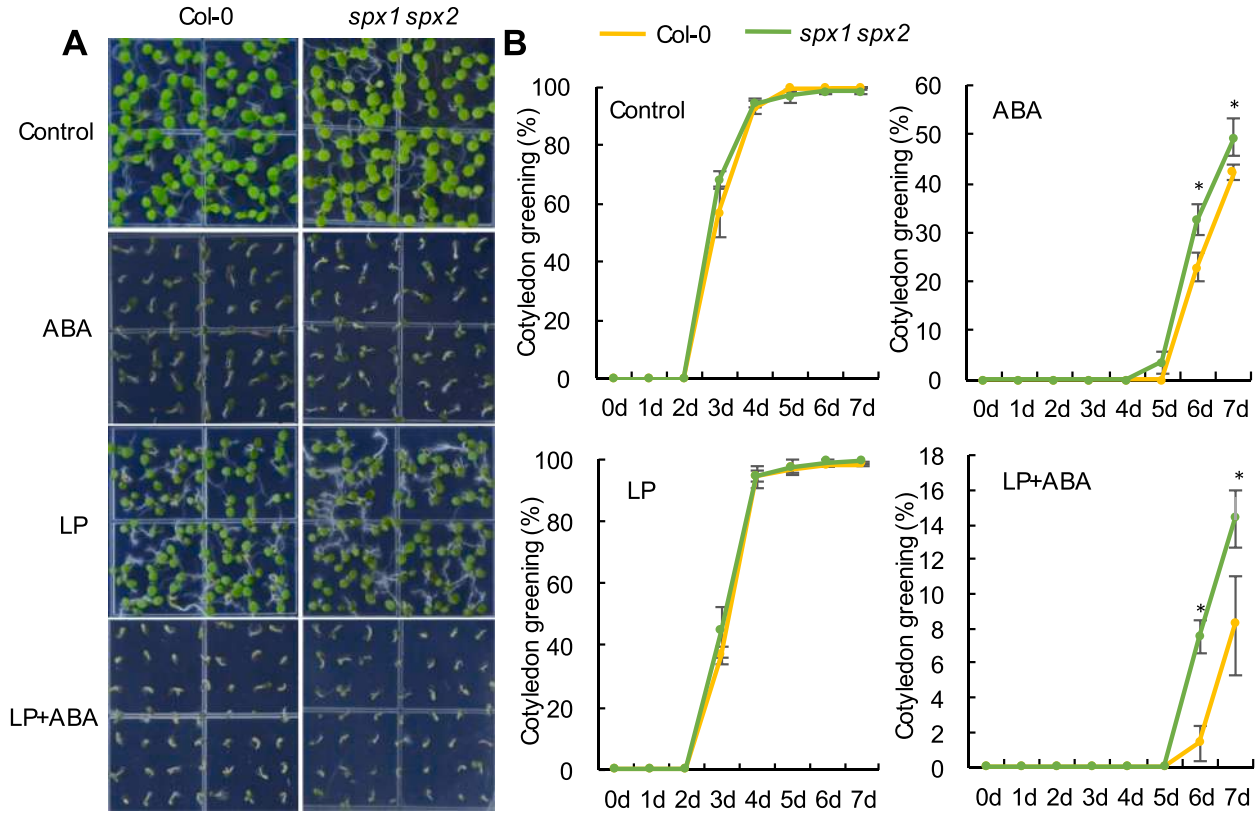

Fig. 4. Seed germination of *spx1 spx2* mutant in responses to ABA, LP, and combined LP+ABA treatments. (A) Seed germination phenotypes of *spx1 spx2* mutant under treatments of ABA, LP and combined LP+ABA. (B) Cotyledon greening rates of *spx1 spx2* seeds under treatments of ABA, LP, and combined LP+ABA. Seeds were germinated and grown under different treatments for seven days (ABA: 1/2 MS with 0.5 μM ABA; LP: 1/2 MS with 10 μM Pi; LP+ABA: 1/2 MS with 10 μM Pi and 0.5 μM ABA). Values are means ± SD of three replicates (at least 40 seeds were used for each replicate). Asterisk indicates a significant difference when compared with that of Col-0 by using Student's *t*-test (*P < 0.05).

3.5. Modulation of PHR1 expression alters stomatal aperture in response to ABA

As ABA signaling plays a central role in regulating stomatal aperture, we investigated the effect of *PHR1* expression on ABA-induced stomatal closure using *PHR1*-knockout mutants and *PHR1*-overexpression plants. Stomatal aperture in the epidermal peels of these mutants was measured after ABA treatment. Under control conditions, the stomatal aperture of *phr1* and *phr1 phl1* mutants was not significantly different from WT, but it was significantly smaller than WT after ABA treatment (Fig. 5A-B). Conversely, the stomatal aperture of *PHR1*-overexpression lines was significantly larger than in WT under ABA treatment (Fig. 5D-E). Additionally, a water-loss rate assay showed that detached leaves from *phr1* and *phr1 phl1* mutants lost water significantly more slowly than WT (Fig. 5C). In contrast, the *PHR1*-overexpression lines and *spx1 spx2* mutant exhibited a significantly faster water loss compared to WT (Fig. 5C, F; Fig. S10B). A drought stress assay revealed that the *phr1 phl1* double mutant was more tolerant to drought stress than WT (Fig. S12). These results suggested that PHR1/PHL1 play a negative role in regulating ABA-mediated stomatal aperture and drought tolerance.

3.6. PHR1 and PHL1 mutations alter sensitivity to salt and osmotic stresses in seed germination

Since ABA plays a pivotal role in salt and osmotic stress responses, we investigated the seed germination rates and cotyledon greening rates of knockout mutants of *PHR1* and PHL1 under salt and osmotic stresses. Similar to ABA, seed germination was inhibited by salt stress (140 mM NaCl) and osmotic stress (300 mM mannitol). The seed germination rates of *phr1*, *phl1*, and *phr1 phl1* mutants were significantly lower than WT under both salt and osmotic stress (Fig. 6; Fig. S13A). Under combined LP and salt (LP+NaCl), and combined LP and osmotic (LP+mannitol) conditions, the seed germination and cotyledon greening rates of these mutants also dropped considerably to WT (Fig. 6; Fig. S13A). The *phr1 phl1* double mutants showed extreme sensitivity under LP+NaCl, with the cotyledon greening rate of WT being four times higher than that of *phr1 phl1* at day 7 after sowing (Fig. 6).

The seed germination and cotyledon greening rates of *PHR1* and *PHL1*-overexpression lines were also significantly higher than WT under salt and osmotic stress (Fig. 7; Fig. S13B-C), similar to the results of ABA treatment (Figs. 2–3). Overexpression of *PHR1* or *PHL1* significantly enhanced seed germination and cotyledon greening rates under LP+NaCl and LP+mannitol treatments (Fig. 7; Fig. S13B-C). These findings suggest that PHR1 and PHL1 regulate seed germination in response to salt and osmotic stress, possibly in an ABA-dependent manner.

3.7. SPX1/SPX2 knockouts reduce salt and osmotic stress sensitivity

We also analyzed seed germination and cotyledon greening in the *spx1 spx2* double mutant under salt and osmotic stress. Similar to the *PHR1*- and *PHL1*-overexpression lines, the seed germination and cotyledon greening rate of *spx1 spx2* was significantly higher than those of WT under salt and osmotic stress, as well as under LP+NaCl and LP+mannitol treatments (Fig. 8; Fig. S13D). Notably, the cotyledon greening rate of WT under LP+mannitol treatment was much lower than that under mannitol alone, whereas the cotyledon greening rate of *spx1 spx2* remained similar between LP+mannitol and mannitol treatments

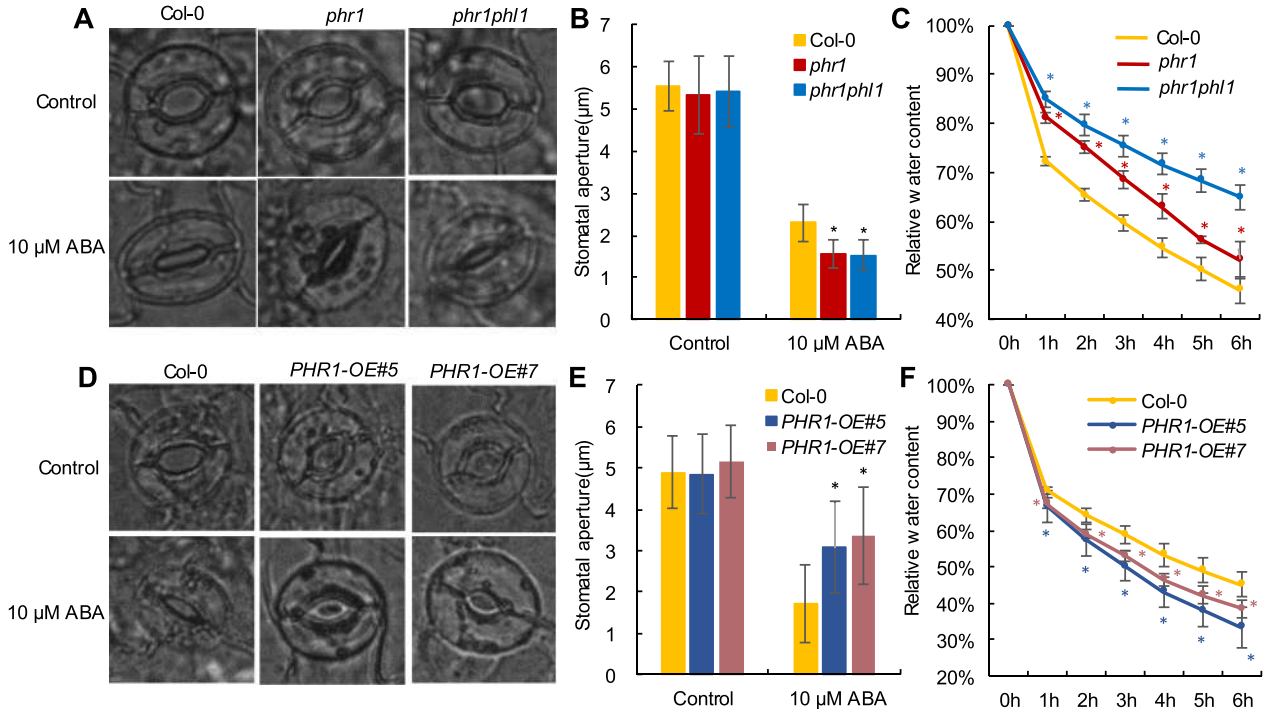

Fig. 5. Stomatal apertures and water loss rates of *phr1*, *phr1 phl1*, *PHR1*-overexpression lines. (A-B) Stomatal apertures under 10 μM ABA treatment of *phr1* and *phr1 phl1* mutants. (C) Leaf water loss rates of *phr1* and *phr1 phl1* mutants within 6 hours. (D-E) Stomatal apertures under 10 μM ABA treatment of *PHR1*-overexpression plants. (F) Leaf water loss rates of *PHR1*-overexpression plants within 6 hours. Leaves from 3-week-old plants were detached and dehydrated, and relative water content was calculated at each indicated time point. Values are means ± SD of three replicate. Asterisk indicates a significant difference when compared with that of Col-0 by using Student's *t*-test (*P < 0.05).

(Fig. 8). This suggests that the knockout mutation of *SPX1* and *SPX2* enhanced resistance to combined LP+mannitol stress during seed germination. These results indicated that SPX1 and SPX2 regulate seed germination in response to salt and osmotic stress, likely by repressing PHR1/PHL1-mediated ABA signaling.

3.8. *PHR1 and PHL1 regulate the expression of genes associated with ABA signaling and stress responses*

Since PHR1 and PHL1 are involved in regulating plant responses to ABA, salt and osmotic stresses, we assumed that ABA, salt, and osmotic-responsive genes are differentially expressed in *phr1* and *phr1 phl1* knockout mutants. Transcriptome analyses of these mutants and Pi-starvation-responsive genes identified through microarray (Bustos et al., 2010) were analyzed for further investigation (Table S2-S7). The transcriptome data revealed that 2727 genes in shoots and 442 genes in roots were commonly differentially expressed in *phr1* and *phr1 phl1* (Table S8-S9). Notably, most of these common genes responded to Pi deficiency (Fig. 9A; Fig. S14A). Gene ontology (GO) term enrichment analysis of the common DEGs in *phr1* and *phr1 phl1* showed significant enrichment in 444 terms in shoots and 115 in roots related to biological processes (adjusted P-value<0.05) (Table S10-S11). A group of GO terms were enriched in both shoots and roots, including response to osmotic stress, water deprivation, oxidative stress, secondary metabolism, and organic acid metabolism (Fig. 9B; Fig. S14B). In shoots, several common DEGs were associated with ABA metabolism, transport, and signaling, and/or responded to ABA. Most of these genes were repressed in *phr1* and *phr1 phl1* but induced by Pi deficiency (Fig. 9C). Notably, 41 % of these genes (31/76) contain P1BS in their 2.0 kb promoters (Fig. 9C; Table S12). In roots, at least 10 common DEGs were linked to ABA metabolism, transport or signaling, and 4 of these genes contained P1BS in their 2.0 kb promoters, including *ADH1*, *AEP2* and *HAI1* (Fig. S14C). These findings suggest that PHR1/PHL1 may directly regulate the expression of genes involved in ABA metabolism, transport, or signaling.

Among the common DEGs in shoots of *phr1* and *phr1 phl1*, at least 107, 82, and 77 genes were associated with osmotic, water, and salt stress responses, respectively (Fig. S15A; Table S13-S15). Nearly half of these genes contain P1BS in their 2.0 kb promoters, including 40 genes linked to osmotic stress, 36 to water stress, and 35 to salt stress (Fig. S16-S18). Notably, 8 genes were shared among ABA metabolism/signaling, osmotic stress response, water stress response, and salt stress response, including *NCED3*, *BGLU18*, *OST1*, *HD2C*, *RD20*, *LTP4*, *ESL1*, and *ERD6* (Fig. S14B).

We further selected 8 ABA-related genes (*HAI1*, *HAI2*, *CYP707A2*, *RD20*, *KIN1*, *NAC019*, *ESL1*, and *EXPA1*) to analyze their expression in the *phr1 phl1* mutant and PHR1-overexpression plants. All these genes were significantly influenced by the modulation of *PHR1/PHL1* (Fig. 10). For example, the expression of *HAI1*, *HAI2*, *CYP707A2*, and *RD20* was down-regulated in the *phr1 phl1* mutant and up-regulated in *PHR1*-overexpression plants; while the expression of *EXPA1* was up-regulated in mutant but down-regulated in *PHR1*-overexpression lines (Fig. 10). These results were largely consistent with the microarray data (Fig. 9C), and suggest that ABA signaling is altered by the modulation of *PHR1/PHL1*.

3.9. *PHR1 orthologs rescue ABA sensitivity in phr1 phl1 mutants*

To determine whether the homologs of PHR1 in other plants play a conserved role in ABA signaling, we generated the complementation lines of *phr1* or *phr1 phl1* using the coding sequences of *CrPSR1* (Cre12.g495100) from *Chlamydomonas reinhardtii* (Bajhaiya et al., 2016), *MpPHR1* (Mapoly0003s0147) from *Marchantia polymorpha* (Rico-Resendiz et al., 2020), and *OsPHR2* (LOC_Os07g25710) from rice (Zhou et al., 2008), all driven by the *Arabidopsis PHR1* promoter. Interestingly, the ABA-sensitive seed germination phenotype of the *phr1*

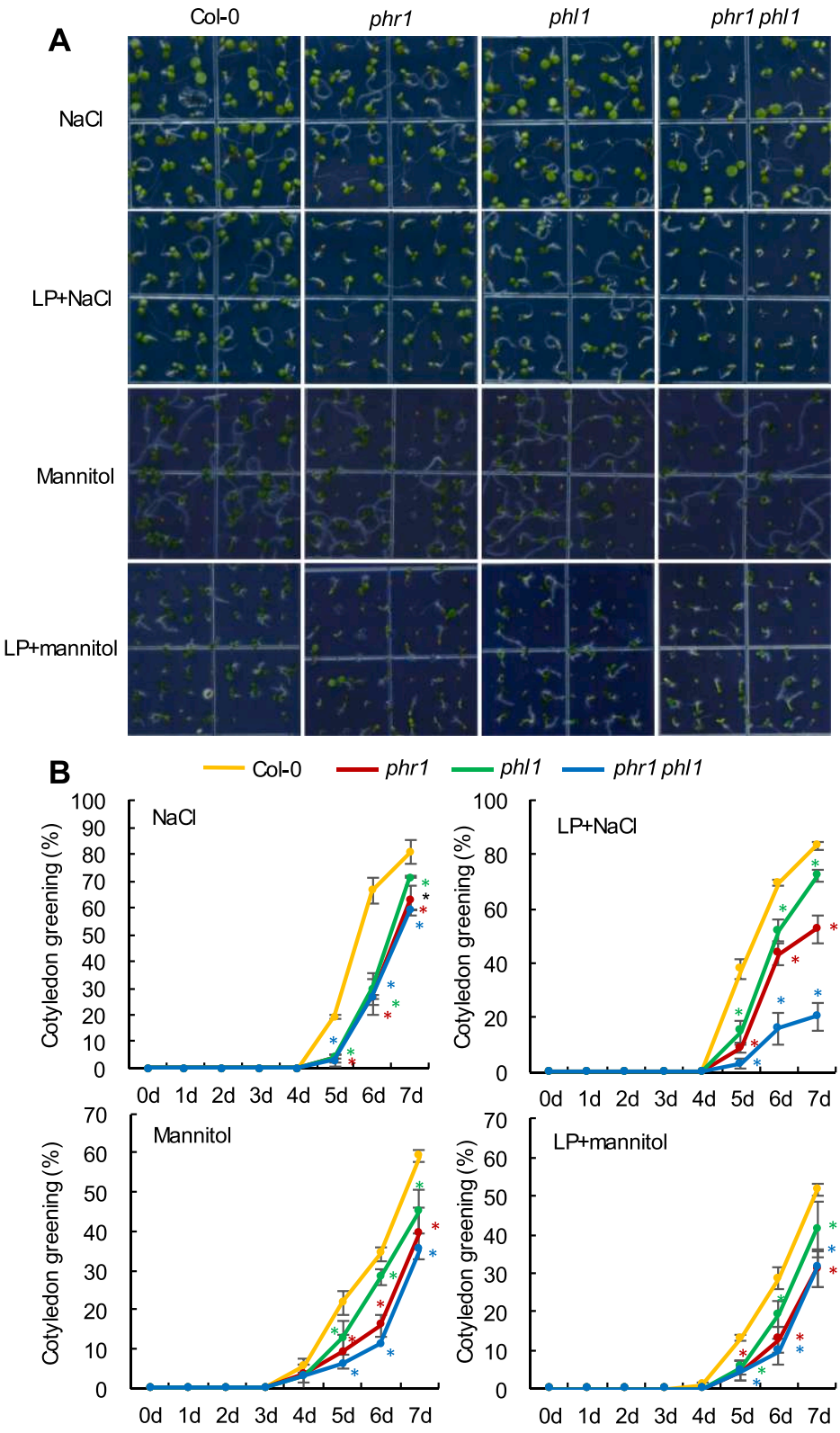

Fig. 6. Seed germination of *phr1*, *phl1*, and *phr1 phl1* mutants in responses to salt, mannitol, combined LP+salt, and combined LP+mannitol stresses. (A) Seed germination phenotypes of *phr1*, *phl1*, and *phr1 phl1* mutants under different treatments. (B) Cotyledon greening rates of *phr1*, *phl1*, and *phr1 phl1* mutants under different treatments. Seeds were germinated and grown under different treatments for seven days (NaCl: 140 mM NaCl; LP+NaCl: 10 μM Pi+140 mM NaCl; Mannitol: 300 mM mannitol; LP+mannitol: 10 μM Pi+300 mM mannitol). Values are means ± SD of three replicates (at least 40 seeds were used for each replicate). Asterisk indicates a significant difference when compared with that of Col-0 by using Student's *t*-test (*P < 0.05).

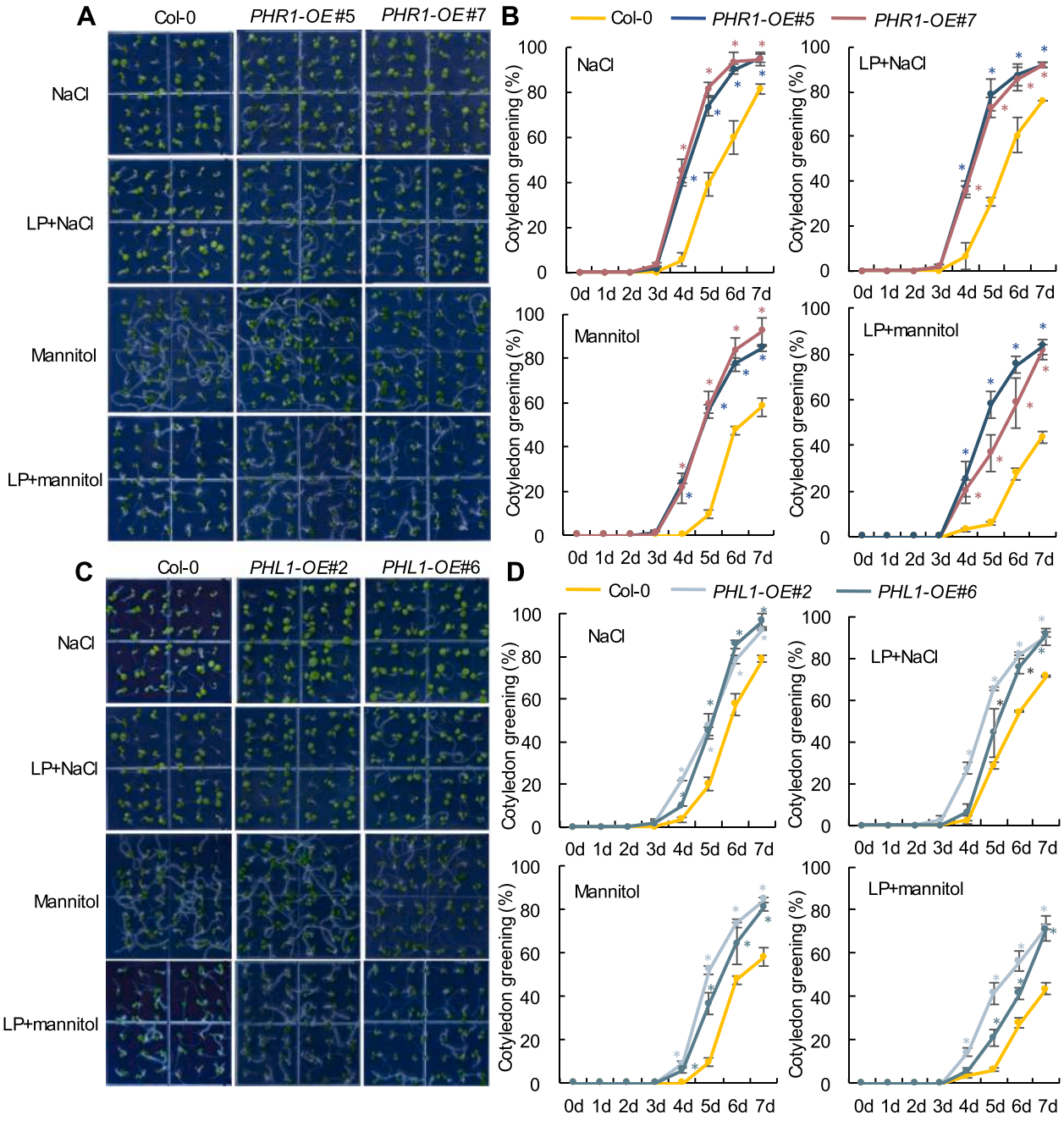

Fig. 7. Seed germination of *PHR1*-overexpression plants, and *PHL1*-overexpression plants in responses to salt, mannitol, combined LP+salt, and combined LP+mannitol stresses. (A) Seed germination phenotypes of *PHR1*-overexpression plants under different treatments. (B) Cotyledon greening rates of *PHR1*-overexpression plants under different treatments. (C) Seed germination phenotypes of *PHL1*-overexpression plants under different treatments. (D) Cotyledon greening rates of *PHL1*-overexpression plants under different treatments. Seeds were germinated and grown under different treatments for seven days (NaCl: 140 mM NaCl; LP+NaCl: 10 μM Pi+140 mM NaCl; Mannitol: 300 mM mannitol; LP+mannitol: 10 μM Pi+300 mM mannitol). Values are means ± SD of three replicates (at least 40 seeds were used for each replicate). Asterisk indicates a significant difference when compared with that of Col-0 by using Student's *t*-test (*P < 0.05).

or *phr1 phl1* mutants was rescued by *CrPSR1*, *MpPHR1* or *OsPHR2* driven by the *AtPHR1* promoter (Fig. 11; Fig. S19). These findings suggest that homologs of PHR1 in other plant species may function similarly to *Arabidopsis* PHR1/PHL1 and play a conserved role in regulating ABA signaling.

4. Discussion

Arabidopsis PHR1 and PHL1 are core transcription factors involved in regulating Pi starvation responses by controlling a group of PSR genes (Bustos et al., 2010). They have also been implicated in plant resistance to various environmental stresses, including high light stress, hypoxia, and pathogen infection (Aleksza et al., 2017; Klecker et al., 2014; Nilsson et al., 2012; Scheible et al., 2023; Castrillo et al., 2017). ABA is a key plant hormone that plays a crucial role in abiotic stress tolerance by inhibiting seed germination, preventing early seedling development, and mediating stomatal closure. In this study, PHR1 and PHL1 were found to be essential for seed germination and early seedling development under LP conditions (Fig. 1). PHR1 and PHL1 appear to negatively regulate ABA signaling, as *PHR1/PHL1* loss of function mutants show

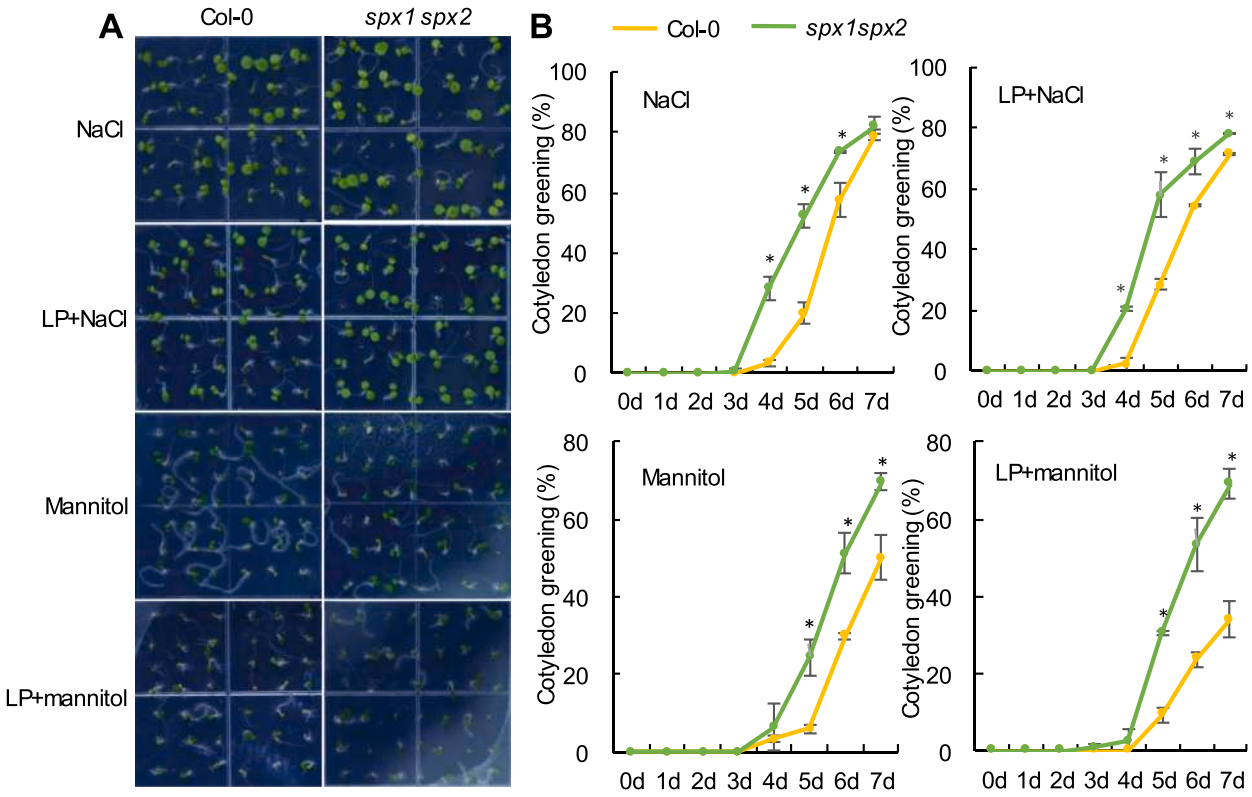

Fig. 8. Seed germination of *spx1 spx2* mutant in responses to salt, mannitol, combined LP+salt, and combined LP+mannitol stresses. (A) Seed germination phenotypes of *spx1 spx2* mutant under different treatments. (B) Cotyledon greening rates of *spx1 spx2* mutant under different treatments. Seeds were germinated and grown under different treatments for seven days (NaCl: 140 mM NaCl; LP+NaCl: 10 μM Pi+140 mM NaCl; Mannitol: 300 mM mannitol; LP+mannitol: 10 μM Pi+300 mM mannitol). Values are means ± SD of three replicates (at least 40 seeds were used for each replicate). Asterisk indicates a significant difference when compared with that of Col-0 by using Student's *t*-test (*P < 0.05).

increased sensitivity to ABA in seed germination, early seedling development, and stomatal aperture (Fig. 1; Fig. 5A-C), while *PHR1/PHL1* overexpression decreases ABA sensitivity (Figs. 2–3; Fig. 5D-F). Since ABA is critical in mediating salt stress- and osmotic stress-mediated inhibition of seed germination and early seedling development (Ali et al., 2020; Nakashima and Yamaguchi-Shinozaki, 2013), PHR1 and PHL1 positively regulate seed germination under these stresses. The loss of function mutants of *PHR1/PHL1* is sensitive to salt and osmotic stresses (Fig. 6), while overexpression of *PHR1/PHL1* confers salt and osmotic stress tolerance in seed germination and early seedling development (Fig. 7). Similarly, knockout mutations of *SPX1* and *SPX2*, which enhance the activities of PHR1 and PHL1 (Puga et al., 2014), reduced sensitivity to ABA, salt and osmotic stresses during seed germination and early seedling development (Fig. 4; Fig. 8). Thus, PHR1 and PHL1 may link between Pi nutrition and ABA, salt and osmotic stress responses in plants.

The interaction between Pi nutrition and ABA signaling has gained attention. ABA levels have been found to increase under Pi deficiency in plants such as *Arabidopsis*, soybean and castor bean (Jaschke et al., 1997; Zhang et al., 2022; Castro-Valdecantos et al., 2023). Numerous ABA- and stress-responsive genes are influenced by Pi deprivation (Scheible et al., 2023; Woo et al., 2012). ABI5, a key component in ABA signaling, has been suggested to regulate Pi uptake by regulating the expression of PSR genes like *PHT1;5* (Lei et al., 2022; Zhang et al., 2022). In this study, PHR1 and PHL1 were found to play negative roles in ABA signaling, possibly by regulating a group of genes involved in ABA metabolism and signaling (Fig. 9). These potential target genes include *CYP707A2*, which encodes a protein with ABA 8′-hydroxylase activity that regulates ABA catabolism and seed dormancy (Okamoto et al., 2006), and *ABCG25*, an ABA exporter involved in long-distance ABA transport and endosperm export (Kang et al., 2015; Yang et al., 2024). Notably, the expression of six out of the nine clades A PP2C genes (*HAI1*, *HAI2*, *ABI1*, *ABI2*, *PP2CA* and *HAB1*), which are core negative regulators in ABA signaling (Chen et al., 2020), was repressed in *phr1* and *phr1 phl1* but was induced by Pi deficiency (Fig. 9C).

On one hand, Pi deficiency may enhance ABA biosynthesis through an unidentified mechanism. On the other hand, ABA signaling is repressed by PHR1 and PHL1, the core components in Pi signaling. Increased ABA levels may result in more stomatal closure and reduce growth rate, which could conserve Pi under limited Pi conditions. However, higher ABA levels may also suppress Pi uptake and translocation, as genes involved in these processes, such as *PHO1*, *PHO1; H1*, and *PHT1;1*, are repressed by ABA in an ABI1-dependent manner (Ribot et al., 2008). Additionally, ABA treatment has been found to reduce shoot Pi concentrations in *Arabidopsis* (Lei et al., 2022). Reduced stomatal opening may decrease transpiration (Radin, 1984), further limiting nutrient uptake, including Pi. Thus, plants may have evolved a mechanism involving PHR1 and PHL1 to repress ABA signaling under Pi starvation, maintaining moderate ABA levels. Interestingly, *PHO1* knockout mutation, a component of the Pi signaling pathways downstream of PHR1/PHL1-miR399-PHO2 cascade (Dai et al., 2024; Yang et al., 2024), confer ABA sensitivity in seed germination and early seedling development (Huang et al., 2017). PHR1 and PHL1 have been implicated as negative regulators in salicylic acid (SA) signaling and positive regulators in jasmonic acid (JA) signaling, as various SA- and JA- responsive genes are affected in the *phr1 phl1* mutant (Castrillo et al., 2017). This study provides evidence of a new role for PHR1 and PHL1 in ABA signaling, though further studies are needed to determine whether PHR1 and PHL1 directly regulate genes related to ABA metabolism or signaling through molecular and genetic mechanisms.

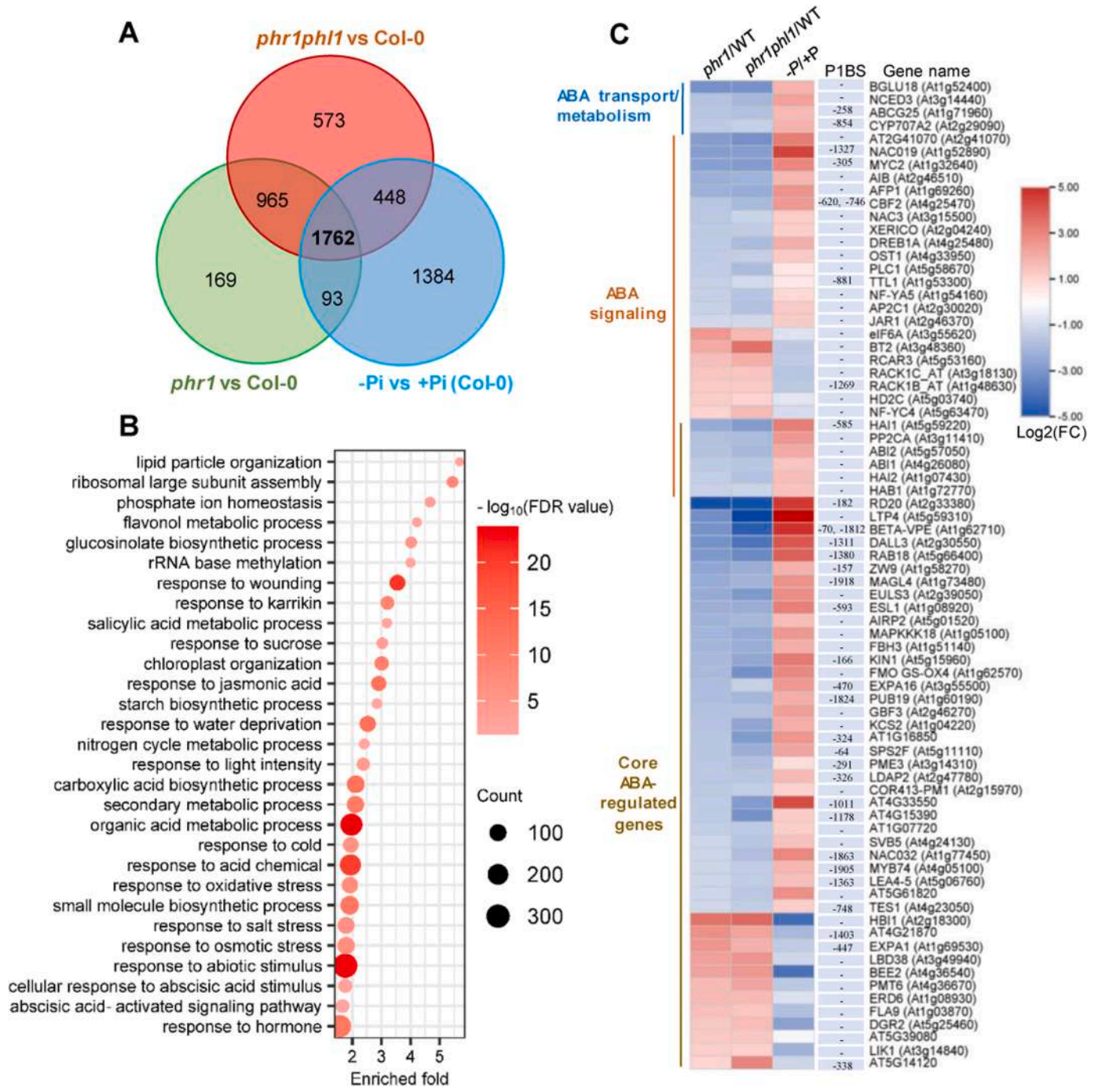

Fig. 9. DEG numbers and representative enriched GO terms in shoots of *phr1* and *phr1 phl1* mutants. (A) Venn diagram representing the overlapped DEGs shared by *phr1* mutant, *phr1 phl1* mutant, and wild-type Pi deficiency. DEGs were revealed by microarray (Fold change > 2, adjusted p-value < 0.05). (B) Representative GO terms significantly enriched for the common DEGs of *phr1* and *phr1 phl1* mutants. (C) The expression of genes related to ABA transport, metabolism, and signaling, and the expression of some core ABA-regulated genes in *phr1* and *phr1 phl1* mutants and in response to Pi deficiency. Red color means up-regulation, and blue color means down-regulation. The presentation and location of PHR1-binding site (P1BS, GNATATNC) in the 2.0 kb promoter regions of these genes were shown. "-" means non-presentation.

In this study, PHR1 and PHL1 were found to be important for seed germination and seedling development under LP, salt, osmotic, combined LP and salt, and combined LP and osmotic stress conditions. Knockout mutants were sensitive to these stresses (Fig. 1; Fig. 6), whereas overexpression of *PHR1* or *PHL1*, as well as mutations in *SPX1* and *SPX2*, promoted seed germination and early seedling development under these conditions (Figs. 2–4; Figs. 7–8). The increased tolerance of plants overexpressing *PHR1*- or *PHL1* to LP, salt, osmotic, and combined stresses during seed germination may be linked to PHR1/PHL1-mediated repression of ABA signaling. Other transcription factors have also been shown to positively regulate seed germination and seedling growth in response to salinity and osmotic stress by repressing ABA signaling. For example, the MYB-like transcription factor DIVARICATA1 (DIV1) promotes seed germination under salinity, osmotic stress, and ABA by directly regulating genes like *DOGL3*, a positive regulator of ABA signaling in *Arabidopsis* (Zhang et al., 2024). Similarly, in rice *SALT AND ABA RESPONSE ERF 1* (*OsSAE1*), a member of the AP2 transcription factor family, enhance salt tolerance by repressing the expression of

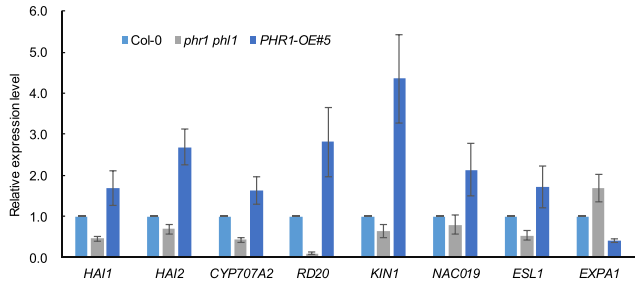

Fig. 10. qRT-PCR analysis of the expression of ABA-related genes in *phr1phl1* mutant and *PHR1*-overexpression plants. Expression levels were normalized to 1.0 in Col-0. Two-week old seedlings were used for the qRT-PCR assay. Values are means ± SD of three replicates.

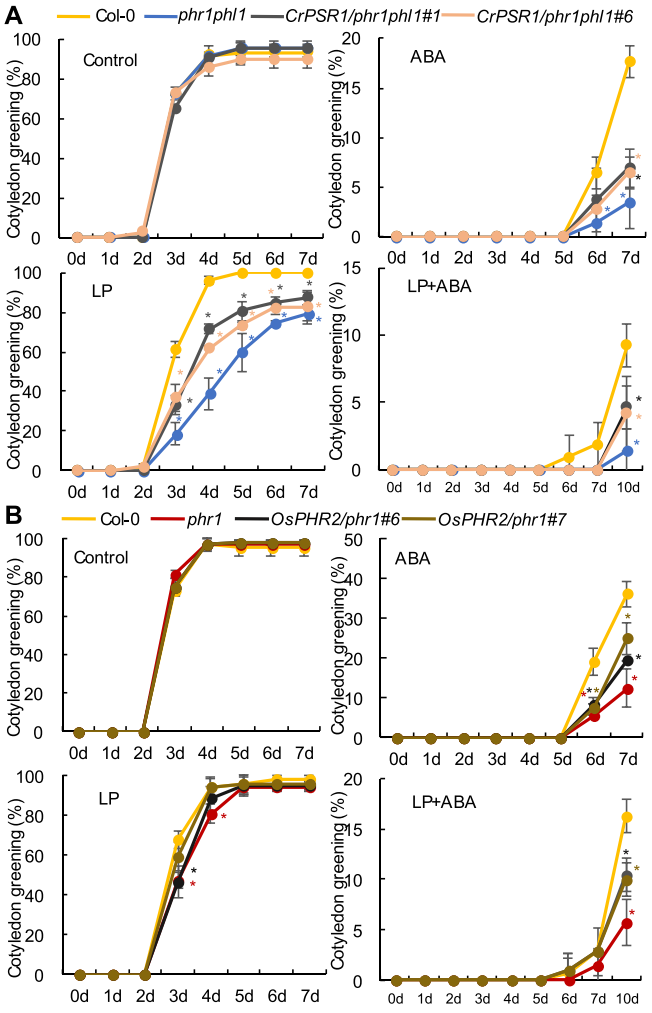

Fig. 11. Seed germination of Col-0 and complementation lines (*pPHR1:CrPSR1/phr1phl1* and *pPHR1:OsPHR2/phr1*) in responses to ABA, LP, and combined LP+ABA treatments. (A) Phenotypes of seed germination of Col-0 and complementation lines under ABA, LP and combined LP+ABA treatments. (B) Cotyledon greening rates of Col-0 and *PHR* complementation lines. Seeds were germinated and grown under different treatments for 7 or 10 days (ABA: 1/2 MS with 0.5 μM ABA; LP: 1/2 MS with 10 μM Pi; LP+ABA: 1/2 MS with 10 μM Pi and 0.5 μM ABA). Values are means ± SD of three replicates (at least 40 seeds were used for each replicate). Asterisk indicates a significant difference when compared with that of Col-0 by using Student's *t*-test (*P < 0.05).

OsABI5, a positive regulator of ABA signaling that negatively affects salts stress tolerance in rice seedlings (Li et al., 2022).

It is widely considered that increasing ABA biosynthesis or signaling can improve plant tolerance to drought (Xu et al., 2021; Zeng et al., 2022; Zhao et al., 2016). Pi in soils depends on water-filled pores and occurs through mass flow and diffusion; thus, reduced soil moisture decreases Pi availability (Li et al., 2024; Pandey et al., 2024). This makes combined LP and drought stresses common in soil. Interestingly, we found that *phr1 phl1* double mutant exhibits increased drought tolerance (Fig. S12), likely due to enhanced ABA-mediated stomatal closure and less water loss (Fig. 5A-C). It is possible that PHR1 and PHL1 also play roles in resistance to the combined LP and drought, combined LP and salt, and other environmental stresses, which warrant further investigation. Additionally, *PHR1* homologs from diverse species, including chlorophyte algae (i.e., *Chlamydomonas reinhardtii CsPSR1*) (Bajhaiya et al., 2016), non-vascular bryophyte (i.e., liverwort *MpPHR1*) (Rico-Resendiz et al., 2020), and monocotyledonous plant (i.e., rice *OsPHR2*) (Zhou et al., 2008), were able to rescue the ABA sensitive phenotype of *phr1* mutants (Fig. 11; Fig. S19). It has been suggested that PHR1 homologs including CsPSR1, MpPHR1 and OsPHR2 can also recognize P1BS element in the promoters of Pi-responsive genes (Bajhaiya et al., 2016; Rico-Resendiz et al., 2020; Zhou et al., 2008). Thus, it is possible that PHR1 homologs in other plants may also regulate the expression of ABA-related genes by binding to the P1BS element in their promoters. It has been well documented that the core ABA signaling components also occurs in algae and bryophytes (Komatsu et al., 2020). Taken together, the roles of PHR1/PHL1 and their homologs in regulating ABA signaling appear to be conserved across plant species.

5. Conclusion

It was concluded that knockout mutation of *PHR1* and *PHL1* leads to increased ABA sensitivity during seed germination, early seedling development, and stomatal aperture, while overexpression of *PHR1* or *PHL1* reduces ABA sensitivity. These knockout mutations also heighten sensitivity to salt and osmotic stresses during seed germination and early seedling development, whereas overexpression of *PHR1* or *PHL1* decreases this sensitivity. Additionally, PHR1 and PHL1 regulate seed germination under combined LP and salt as well as LP and osmotic stress conditions. A group of genes associated with ABA metabolism, transport, signalling, and response to osmotic, water, and salt stress may be regulated by PHR1 and PHL1. Furthermore, homologs of PHR1 from other plants may play a similar role in regulating ABA signalling. These results indicate that PHR1 and its homologs are involved in ABA signalling and regulate seed germination under salt and osmotic stresses. These results also reveal a new role for PHR1 and its homologs in the linking of Pi nutrition and ABA signalling. In the future, modulating the expression of *PHR1/PHL1* and their homologs in crops could enhance seed germination and improve Pi acquisition under combined LP and salt/osmotic stress conditions.

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Author statement

Houqing Zeng conceived the study. Huiying Chen, Yifan Wang, Jia Du, Kexin Chao, and Zitong Wang performed the experiments. Huiying Chen and Houqing Zeng analyzed the data. Houqing Zeng and Huiying Chen wrote the manuscript. Shahid Ali revised the manuscript. All the authors have approved the contents of the manuscript.

CRediT authorship contribution statement

Kexin Chao: Data curation. **Zitong Wang:** Data curation. **Jia Du:** Investigation, Data curation. **Yifan Wang:** Investigation, Data curation. **Shahid Ali:** Writing – review & editing. **Houqing Zeng:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Formal analysis, Conceptualization. **Huiying Chen:** Writing – original draft, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Houqing Zeng reports financial support was provided by National Natural Science Foundation of China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.plantsci.2025.112389.

Data availability

Data will be made available on request.

References

M.D. Abramoff, P.J. Magalhães, S.J. Ram, Image processing with ImageJ, Biophotonics Int. 11 (7) (2004) 36–41.

D. Aleksza, G.V. Horváth, G. Sándor, L. Szabados, Proline accumulation is regulated by transcription factors associated with phosphate starvation, Plant Physiol. 175 (1) (2017) 555–567, https://doi.org/10.1104/pp.17.00791.

F. Ali, G. Qanmber, F. Li, Z. Wang, Updated role of ABA in seed maturation, dormancy, and germination, J. Adv. Res. 35 (2022) 199–214, https://doi.org/10.1016/j.jare.2021.03.011.

S. Ali, K. Hayat, A. Iqbal, L. Xie, Implications of abscisic acid in the drought stress tolerance of plants, Agronomy 10 (9) (2020) 1323.

A.K. Bajhaiya, A.P. Dean, L.A. Zeef, R.E. Webster, J.K. Pittman, PSR1 is a global transcriptional regulator of phosphorus deficiency responses and carbon storage metabolism in chlamydomonas reinhardtii, Plant Physiol. 170 (3) (2016) 1216–1234, https://doi.org/10.1104/pp.15.01907.

R. Bustos, G. Castrillo, F. Linhares, M.I. Puga, V. Rubio, J. Perez-Perez, R. Solano, A. Leyva, J. Paz-Ares, A central regulatory system largely controls transcriptional activation and repression responses to phosphate starvation in Arabidopsis, PLoS Genet 6 (9) (2010) e1001102, https://doi.org/10.1371/journal.pgen.1001102.

G. Castrillo, P.J.P.L. Teixeira, S.H. Paredes, T.F. Law, L. de Lorenzo, M.E. Feltcher, O. M. Finkel, N.W. Breakfield, P. Mieczkowski, C.D. Jones, J. Paz-Ares, J.L. Dangl, Root microbiota drive direct integration of phosphate stress and immunity, Nature 543 (7646) (2017) 513–518, https://doi.org/10.1038/nature21417.

P. Castro-Valdecantos, P.A. Martínez-Melgarejo, F. Pérez-Alfocea, J. Tian, I.C. Dodd, Stem girdling enhances ABA-induced stomatal closure of phosphorus-deprived soybean plants, Environ. Exp. Bot. 208 (2023) 105266, https://doi.org/10.1016/j.envexpbot.2023.105266.

K. Chen, G.J. Li, R.A. Bressan, C.P. Song, J.K. Zhu, Y. Zhao, Abscisic acid dynamics, signaling, and functions in plants, J. Integr. Plant Biol. 62 (1) (2020) 25–54, https://doi.org/10.1111/jipb.12899.

S.J. Clough, A.F. Bent, Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana, Plant J. 16 (6) (1998) 735–743, https://doi.org/10.1046/j.1365-313x.1998.00343.x.

S.H. Dai, H.Y. Chen, Y.T. Shi, X.L. Xiao, L. Xu, C. Qin, Y.Y. Zhu, K.K. Yi, M.G. Lei, H. Q. Zeng, PHOSPHATE1-mediated phosphate translocation from roots to shoots regulates floral transition in plants, J. Exp. Bot. 75 (16) (2024) 5054–5075, https://doi.org/10.1093/jxb/erae222.

M. Ding, Y. Zhu, T. Kinoshita, Stomatal properties of Arabidopsis cauline and rice flag leaves and their contributions to seed production and grain yield, J. Exp. Bot. 74 (6) (2023) 1957–1973, https://doi.org/10.1093/jxb/erac492.

J. Dong, G. Ma, L. Sui, M. Wei, V. Satheesh, R. Zhang, S. Ge, J. Li, T.E. Zhang, C. Wittwer, H.J. Jessen, H. Zhang, G.Y. An, D.Y. Chao, D. Liu, M. Lei, Inositol pyrophosphate InsP8 acts as an intracellular phosphate signal in Arabidopsis, Mol. Plant 12 (11) (2019) 1463–1473, https://doi.org/10.1016/j.molp.2019.08.002.

R. Finkelstein, Abscisic acid synthesis and response, Arab. Book / Am. Soc. Plant Biol. 11 (2013) e0166, https://doi.org/10.1199/table0166.

H. Fujii, P.E. Verslues, J.K. Zhu, Arabidopsis decuple mutant reveals the importance of SnRK2 kinases in osmotic stress responses in vivo, Proc. Natl. Acad. Sci. USA 108 (4) (2011) 1717–1722, https://doi.org/10.1073/pnas.1018367108.

M. Fujita, Y. Fujita, K. Maruyama, M. Seki, K. Hiratsu, M. Ohme-Takagi, L.S.P. Tran, K. Yamaguchi-Shinozaki, K. Shinozaki, A dehydration-induced NAC protein, RD26, is involved in a novel ABA-dependent stress-signaling pathway, Plant J. 39 (6) (2004) 863–876.

Y. Fujita, T. Yoshida, K. Yamaguchi-Shinozaki, Pivotal role of the AREB/ABF-SnRK2 pathway in ABRE-mediated transcription in response to osmotic stress in plants, Physiol. Plant. 147 (1) (2013) 15–27, https://doi.org/10.1111/j.1399-3054.2012.01635.x.

M. Gu, A. Chen, S. Sun, G. Xu, Complex regulation of plant phosphate transporters and the gap between molecular mechanisms and practical application: what are missing? Mol. Plant 9 (3) (2016) 396–416, https://doi.org/10.1016/j.molp.2015.12.012.

P.K. Hsu, G. Dubeaux, Y. Takahashi, J.I. Schroeder, Signaling mechanisms in abscisic acid-mediated stomatal closure, Plant J. 105 (2) (2021) 307–321, https://doi.org/10.1111/tpj.15067.

Y. Huang, M.M. Sun, Q. Ye, X.Q. Wu, W.H. Wu, Y.F. Chen, Abscisic acid modulates seed germination via ABA INSENSITIVE5-mediated PHOSPHATE1, Plant Physiol. 175 (4) (2017) 1661–1668, https://doi.org/10.1104/pp.17.00164.

W.D. Jaschke, A.D. Peuke, J.S. Pate, W. Hartung, Transport, synthesis and catabolism of abscisic acid (ABA) in intact plants of castor bean (Ricinus communis L.) under phosphate deficiency and moderate salinity, J. Exp. Bot. 48 (9) (1997) 1737–1747, https://doi.org/10.1093/jxb/48.9.1737.

X. Jia, L. Wang, L. Nussaume, K. Yi, Cracking the code of plant central phosphate signaling, Trends Plant Sci. 28 (3) (2023) 267–270, https://doi.org/10.1016/j.tplants.2022.12.008.

X. Jia, L. Wang, H. Zeng, K. Yi, Insights of intracellular/intercellular phosphate transport and signaling in unicellular green algae and multicellular land plants, N. Phytol. 232 (4) (2021) 1566–1571, https://doi.org/10.1111/nph.17716.

Y. Jiang, G. Liang, D. Yu, Activated expression of WRKY57 confers drought tolerance in Arabidopsis, Mol. Plant 5 (6) (2012) 1375–1388, https://doi.org/10.1093/mp/sss080.

J. Kang, S. Yim, H. Choi, A. Kim, K.P. Lee, L. Lopez-Molina, E. Martinoia, Y. Lee, Abscisic acid transporters cooperate to control seed germination, Nat. Commun. 6 (2015) 8113, https://doi.org/10.1038/ncomms9113.

M. Klecker, P. Gasch, H. Peisker, P. Dormann, H. Schlicke, B. Grimm, A. Mustroph, A shoot-specific hypoxic response of Arabidopsis sheds light on the role of the phosphate-responsive transcription factor PHOSPHATE STARVATION RESPONSE1, Plant Physiol. 165 (2) (2014) 774–790, https://doi.org/10.1104/pp.114.237990.

K. Komatsu, D. Takezawa, Y. Sakata, Decoding ABA and osmostress signalling in plants from an evolutionary point of view, Plant Cell Environ. 43 (12) (2020) 2894–2911, https://doi.org/10.1111/pce.13869.

S.J Lee, J.Y Kang, H.J. Park, M.D. Kim, M.S. Bae, H.I Choi, S.Y. Kim, DREB2C interacts with ABF2, a bZIP protein regulating abscisic acid-responsive gene expression, and its overexpression affects abscisic acid sensitivity, Plant Physiol 153 (2) (2010) 716–727, https://doi.org/10.1104/pp.110.154617.

K.J. Lei, H. Zhou, D.L. Gu, G.Y. An, The involvement of abscisic acid-insensitive mutants in low phosphate stress responses during rhizosphere acidification, anthocyanin accumulation and Pi homeostasis in Arabidopsis, Plant Sci. 322 (2022) 111358, https://doi.org/10.1016/j.plantsci.2022.111358.

G. Li, Y. Wang, K. Fu, C. Li, J. Wei, J. Zhu, Y. Wang, C. Li, C. Li, Split application of phosphorus fertilizer increased wheat grain weight under post-anthesis drought stress by delaying programmed cell death of endosperm, Environ. Exp. Bot. 226 (2024) 105907, https://doi.org/10.1016/j.envexpbot.2024.105907.

X. Li, X. Li, M. Li, Y. Yan, X. Liu, L. Li, Dual function of NAC072 in ABF3-mediated ABA-responsive gene regulation in Arabidopsis, Front. Plant Sci. 7 (2016), https://doi.org/10.3389/fpls.2016.01075.

Y. Li, J. Zhou, Z. Li, J. Qiao, R. Quan, J. Wang, R. Huang, H. Qin, SALT AND ABA RESPONSE ERF1 improves seed germination and salt tolerance by repressing ABA signaling in rice, Plant Physiol. 189 (2) (2022) 1110–1127, https://doi.org/10.1093/plphys/kiac125.

W.Y. Lin, T.K. Huang, T.J. Chiou, Nitrogen limitation adaptation, a target of microRNA827, mediates degradation of plasma membrane-localized phosphate transporters to maintain phosphate homeostasis in Arabidopsis, Plant Cell 25 (10) (2013) 4061–4074, https://doi.org/10.1105/tpc.113.116012.

J. Liu, D. Shu, Z. Tan, M. Ma, N. Guo, S. Gao, G. Duan, B. Kuai, Y. Hu, S. Li, D. Cui, The Arabidopsis IDD14 transcription factor interacts with bZIP-type ABFs/AREBs and cooperatively regulates ABA-mediated drought tolerance, N. Phytol. 236 (3) (2022) 929–942, https://doi.org/10.1111/nph.18381.

J. Liu, L. Yang, M. Luan, Y. Wang, C. Zhang, B. Zhang, J. Shi, F.G. Zhao, W. Lan, S. Luan, A vacuolar phosphate transporter essential for phosphate homeostasis in Arabidopsis, Proc. Natl. Acad. Sci. USA 112 (47) (2015) E6571–E6578, https://doi.org/10.1073/pnas.1514598112.

T.Y. Liu, T.K. Huang, S.Y. Yang, Y.T. Hong, S.M. Huang, F.N. Wang, S.F. Chiang, S. Y. Tsai, W.C. Lu, T.J. Chiou, Identification of plant vacuolar transporters mediating phosphate storage, Nat. Commun. 7 (2016) 11095, https://doi.org/10.1038/ncomms11095.

D.L. Lopez-Arredondo, M.A. Leyva-Gonzalez, S.I. Gonzalez-Morales, J. Lopez-Bucio, L. Herrera-Estrella, Phosphate nutrition: improving low-phosphate tolerance in crops, Annu. Rev. Plant Biol. 65 (2014) 95–123, https://doi.org/10.1146/annurev-arplant-050213-035949.

L. Lopez-Molina, S. Mongrand, N.-H. Chua, A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in Arabidopsis, Proc. Natl. Acad. Sci. 98 (8) (2001) 4782–4787, https://doi.org/10.1073/pnas.081594298.

H. Lu, F. Wang, Y. Wang, R. Lin, Z. Wang, C. Mao, Molecular mechanisms and genetic improvement of low-phosphorus tolerance in rice, Plant Cell Environ. 46 (4) (2023) 1104–1119, https://doi.org/10.1111/pce.14457.
I. Madison, L. Gillan, J. Peace, F. Gabrieli, L. van den Broeck, J.L. Jones, R. Sozzani, Phosphate starvation: response mechanisms and solutions, J. Exp. Bot. 74 (21) (2023) 6417–6430, https://doi.org/10.1093/jxb/erad326.
S. Nakamura, T.J. Lynch, R.R. Finkelstein, Physical interactions between ABA response loci of Arabidopsis, Plant J. 26 (6) (2001) 627–635, https://doi.org/10.1046/j.1365-313x.2001.01069.x.
K. Nakashima, K. Yamaguchi-Shinozaki, ABA signaling in stress-response and seed development, Plant Cell Rep. 32 (7) (2013) 959–970, https://doi.org/10.1007/s00299-013-1418-1.
M. Nezamivand-Chegini, E. Ebrahimie, A. Tahmasebi, A. Moghadam, S. Eshghi, M. Mohammadi-Dehchesmeh, S. Kopriva, A. Niazi, New insights into the evolution of SPX gene family from algae to legumes; a focus on soybean, BMC Genom. 22 (1) (2021) 915, https://doi.org/10.1186/s12864-021-08242-5.
L. Nilsson, M. Lundmark, P.E. Jensen, T.H. Nielsen, The Arabidopsis transcription factor PHR1 is essential for adaptation to high light and retaining functional photosynthesis during phosphate starvation, Physiol. Plant. 144 (1) (2012) 35–47, https://doi.org/10.1111/j.1399-3054.2011.01520.x.
L. Nilsson, R. Müler, T.H. Nielsen, Increased expression of the MYB-related transcription factor, PHR1, leads to enhanced phosphate uptake in Arabidopsis thaliana, Plant, Cell Environ. 30 (12) (2007) 1499–1512, https://doi.org/10.1111/j.1365-3040.2007.01734.x.
M. Okamoto, A. Kuwahara, M. Seo, T. Kushiro, T. Asami, N. Hirai, Y. Kamiya, T. Koshiba, E. Nambara, CYP707A1 and CYP707A2, which encode abscisic Acid 8′-hydroxylases, are indispensable for proper control of seed dormancy and germination in Arabidopsis, Plant Physiol. 141 (1) (2006) 97–107, https://doi.org/10.1104/pp.106.079475.
R. Pandey, S. Sharma, A. Mishra, A.S. Sakhare, S.K. Meena, K. Vengavasi, Dual-nutrient stress tolerance in wheat is regulated by nitrogen and phosphorus uptake, assimilation, reutilization, and differential expression of candidate genes, Plant Soil (2024), https://doi.org/10.1007/s11104-024-06789-2.
B.D. Pant, P. Pant, A. Erban, D. Huhman, J. Kopka, W.R. Scheible, Identification of primary and secondary metabolites with phosphorus status-dependent abundance in Arabidopsis, and of the transcription factor PHR1 as a major regulator of metabolic changes during phosphorus limitation, Plant Cell Environ. 38 (1) (2015) 172–187, https://doi.org/10.1111/pce.12378.
S.H. Park, J.S. Jeong, C.H. Huang, B.S. Park, N.H. Chua, Inositol polyphosphates-regulated polyubiquitination of PHR1 by NLA E3 ligase during phosphate starvation response in Arabidopsis, N. Phytol. 237 (4) (2023) 1215–1228, https://doi.org/10.1111/nph.18621.
M.I. Puga, I. Mateos, R. Charukesi, Z. Wang, J.M. Franco-Zorrilla, L. de Lorenzo, M. L. Irigoyen, S. Masiero, R. Bustos, J. Rodriguez, A. Leyva, V. Rubio, H. Sommer, J. Paz-Ares, SPX1 is a phosphate-dependent inhibitor of phosphate starvation response 1 in Arabidopsis, Proc. Natl. Acad. Sci. USA 111 (41) (2014) 14947–14952, https://doi.org/10.1073/pnas.1404654111.
J.W. Radin, Stomatal responses to water stress and to abscisic acid in phosphorus-deficient cotton plants, Plant Physiol. 76 (2) (1984) 392–394, https://doi.org/10.1104/pp.76.2.392.
K.G. Raghothama, Phosphate acquisition, Annu Rev. Plant Physiol. Plant Mol. Biol. 50 (1999) 665–693, https://doi.org/10.1146/annurev.arplant.50.1.665.
C. Ribot, Y. Wang, Y. Poirier, 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 (5) (2008) 1025–1036, https://doi.org/10.1007/s00425-007-0677-x.
F. Rico-Resendiz, S.A. Cervantes-Perez, A. Espinal-Centeno, M. Dipp-Alvarez, A. Oropeza-Aburto, E. Hurtado-Bautista, A. Cruz-Hernandez, J.L. Bowman, K. Ishizaki, M.A. Arteaga-Vazquez, L. Herrera-Estrella, A. Cruz-Ramirez, Transcriptional and morpho-physiological responses of marchantia polymorpha upon phosphate starvation, Int. J. Mol. Sci. 21 (21) (2020), https://doi.org/10.3390/ijms21218354.
E. Riemer, D. Qiu, D. Laha, R.K. Harmel, P. Gaugler, V. Gaugler, M. Frei, M.R. Hajirezaei, N.P. Laha, L. Krusenbaum, R. Schneider, A. Saiardi, D. Fiedler, H.J. Jessen, G. Schaaf, R.F.H. Giehl, ITPK1 is an InsP(6)/ADP phosphotransferase that controls phosphate signaling in Arabidopsis, Mol. Plant 14 (11) (2021) 1864–1880, https://doi.org/10.1016/j.molp.2021.07.011.
H. Rouached, A. Stefanovic, D. Secco, A. Bulak Arpat, E. Gout, R. Bligny, Y. Poirier, Uncoupling phosphate deficiency from its major effects on growth and transcriptome via PHO1 expression in Arabidopsis, Plant J. 65 (4) (2011) 557–570, https://doi.org/10.1111/j.1365-313X.2010.04442.x.
W. Ruan, M. Guo, X. Wang, Z. Guo, Z. Xu, L. Xu, H. Zhao, H. Sun, C. Yan, K. Yi, Two RING-Finger ubiquitin E3 ligases regulate the degradation of SPX4, an internal phosphate sensor, for phosphate homeostasis and signaling in rice, Mol. Plant 12 (8) (2019) 1060–1074, https://doi.org/10.1016/j.molp.2019.04.003.
V. Rubio, F. Linhares, R. Solano, A.C. Martin, J. Iglesias, A. Leyva, J. Paz-Ares, A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae, Genes Dev. 15 (16) (2001) 2122–2133, https://doi.org/10.1101/gad.204401.
W.R. Scheible, P. Pant, B.D. Pant, N. Krom, R.D. Allen, K.S. Mysore, Elucidating the unknown transcriptional responses and PHR1-mediated biotic and abiotic stress tolerance during phosphorus limitation, J. Exp. Bot. 74 (6) (2023) 2083–2111, https://doi.org/10.1093/jxb/erad009.
L. Sun, L. Song, Y. Zhang, Z. Zheng, D. Liu, Arabidopsis PHL2 and PHR1 act redundantly as the key components of the central regulatory system controlling transcriptional responses to phosphate starvation, Plant Physiol. 170 (1) (2016) 499–514, https://doi.org/10.1104/pp.15.01336.
C.P. Vance, C. Uhde-Stone, D.L. Allan, Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource, N. Phytol. 157 (3) (2003) 423–447, https://doi.org/10.1046/j.1469-8137.2003.00695.x.
J. Waese, J. Fan, A. Pasha, H. Yu, G. Fucile, R. Shi, M. Cumming, L.A. Kelley, M. J. Sternberg, V. Krishnakumar, E. Ferlanti, J. Miller, C. Town, W. Stuerzlinger, N. J. Provart, ePlant: visualizing and exploring multiple levels of data for hypothesis generation in plant biology, Plant Cell 29 (8) (2017) 1806–1821, https://doi.org/10.1105/tpc.17.00073.
X. Wang, C. Guo, J. Peng, C. Li, F. Wan, S. Zhang, Y. Zhou, Y. Yan, L. Qi, K. Sun, S. Yang, Z. Gong, J. Li, ABRE-BINDING FACTORS play a role in the feedback regulation of ABA signaling by mediating rapid ABA induction of ABA co-receptor genes, N. Phytol. 221 (1) (2019) 341–355, https://doi.org/10.1111/nph.15345.
Z. Wang, H.-F. Kuo, T.-J. Chiou, Intracellular phosphate sensing and regulation of phosphate transport systems in plants, Plant Physiol. 187 (4) (2021) 2043–2055, https://doi.org/10.1093/plphys/kiab343.
Z. Wang, Z. Zheng, Y.M. Zhu, S.Y. Kong, D. Liu, PHOSPHATE RESPONSE 1 family members act distinctly to regulate transcriptional responses to phosphate starvation, Plant Physiol. 191 (2) (2023) 1324–1343, https://doi.org/10.1093/plphys/kiac521.
J. Woo, C.R. MacPherson, J. Liu, H. Wang, T. Kiba, M.A. Hannah, X.J. Wang, V.B. Bajic, N.H. Chua, The response and recovery of the Arabidopsis thaliana transcriptome to phosphate starvation, BMC Plant Biol. 12 (1) (2012) 62, https://doi.org/10.1186/1471-2229-12-62.
M. Xu, H. Li, Z.-N. Liu, X.-H. Wang, P. Xu, S.-J. Dai, X. Cao, X.-Y. Cui, The soybean CBL-interacting protein kinase, GmCIPK2, positively regulates drought tolerance and ABA signaling, Plant Physiol. Biochem. 167 (2021) 980–989, https://doi.org/10.1016/j.plaphy.2021.09.026.
Z.Y. Xu, S.Y. Kim, Y. Hyeon do, D.H. Kim, T. Dong, Y. Park, J.B. Jin, S.H. Joo, S.K. Kim, J. C. Hong, D. Hwang, I. Hwang, The Arabidopsis NAC transcription factor ANAC096 cooperates with bZIP-type transcription factors in dehydration and osmotic stress responses, Plant Cell 25 (11) (2013) 4708–4724, https://doi.org/10.1105/tpc.113.119099.
Q. Yang, X. Deng, T. Liu, J. Qian, P. Zhang, E. Zhu, J. Wang, X. Zhu, G. Kudoyarova, J. Zhao, K. Zhang, Abscisic acid root-to-shoot translocation by transporter AtABCG25 mediates stomatal movements in Arabidopsis, Plant Physiol. 195 (1) (2024) 671–684, https://doi.org/10.1093/plphys/kiae073.
S.Y. Yang, W.Y. Lin, Y.M. Hsiao, T.J. Chiou, Milestones in understanding transport, sensing, and signaling of the plant nutrient phosphorus, Plant Cell 36 (5) (2024) 1504–1523, https://doi.org/10.1093/plcell/koad326.
H. Zeng, H. Wu, G. Wang, S. Dai, Q. Zhu, H. Chen, K. Yi, L. Du, Arabidopsis CAMTA3/SR1 is involved in drought stress tolerance and ABA signaling, Plant Sci. 319 (2022) 111250, https://doi.org/10.1016/j.plantsci.2022.111250.
H. Zeng, C. Xia, C. Zhang, L.-Q. Chen, A simplified hydroponic culture of Arabidopsis, Bio-Protoc. 8 (24) (2018) e3121, https://doi.org/10.21769/BioProtoc.3121.
H. Zeng, X. Zhang, X. Zhang, E. Pi, L. Xiao, Y. Zhu, Early transcriptomic response to phosphate deprivation in soybean leaves as revealed by RNA-sequencing, Int J. Mol. Sci. 19 (7) (2018), https://doi.org/10.3390/ijms19072145.
D. Zhang, T. He, X. Wang, C. Zhou, Y. Chen, X. Wang, S. Wang, S. He, Y. Guo, Z. Liu, M. Chen, Transcription factor DIVARICATA1 positively modulates seed germination in response to salinity stress, Plant Physiol. 195 (4) (2024) 2997–3009, https://doi.org/10.1093/plphys/kiae231.
J. Zhang, X. Zhou, Y. Xu, M. Yao, F. Xie, J. Gai, Y. Li, S. Yang, Soybean SPX1 is an important component of the response to phosphate deficiency for phosphorus homeostasis, Plant Sci. 248 (2016) 82–91, https://doi.org/10.1016/j.plantsci.2016.04.010.
Y. Zhang, T.-T. Li, L.-F. Wang, J.-X. Guo, K.-K. Lu, R.-F. Song, J.-X. Zuo, H.-H. Chen, W.-C. Liu, Abscisic acid facilitates phosphate acquisition through the transcription factor ABA INSENSITIVE5 in Arabidopsis, Plant J. 111 (1) (2022) 269–281, https://doi.org/10.1111/tpj.15791.
H. Zhao, K. Nie, H. Zhou, X. Yan, Q. Zhan, Y. Zheng, C.P. Song, ABI5 modulates seed germination via feedback regulation of the expression of the PYR/PYL/RCAR ABA receptor genes, N. Phytol. 228 (2) (2020) 596–608, https://doi.org/10.1111/nph.16713.
Y. Zhao, Z.L. Chan, J.H. Gao, L. Xing, M.J. Cao, C.M. Yu, Y.L. Hu, J. You, H.T. Shi, Y. F. Zhu, Y.H. Gong, Z.X. Mu, H.Q. Wang, X. Deng, P.C. Wang, R.A. Bressan, J.K. Zhu, ABA receptor PYL9 promotes drought resistance and leaf senescence, Proc. Natl. Acad. Sci. USA 113 (7) (2016) 1949–1954, https://doi.org/10.1073/pnas.1522840113.
J. Zhou, Q. Hu, X. Xiao, D. Yao, S. Ge, J. Ye, H. Li, R. Cai, R. Liu, F. Meng, C. Wang, J. K. Zhu, M. Lei, W. Xing, Mechanism of phosphate sensing and signaling revealed by rice SPX1-PHR2 complex structure, Nat. Commun. 12 (1) (2021) 7040, https://doi.org/10.1038/s41467-021-27391-5.
J. Zhou, F. Jiao, Z. Wu, Y. Li, X. Wang, X. He, W. Zhong, P. Wu, OsPHR2 is involved in phosphate-starvation signaling and excessive phosphate accumulation in shoots of plants, Plant Physiol. 146 (4) (2008) 1673–1686.
J. Zhu, K. Lau, R. Puschmann, R.K. Harmel, Y. Zhang, V. Pries, P. Gaugler, L. Broger, A. K. Dutta, H.J. Jessen, G. Schaaf, A.R. Fernie, L.A. Hothorn, D. Fiedler, M. Hothorn, Two bifunctional inositol pyrophosphate kinases/phosphatases control plant phosphate homeostasis, Elife 8 (2019) e43582, https://doi.org/10.7554/eLife.43582.
C. Zimmerli, C. Ribot, A. Vavasseur, H. Bauer, R. Hedrich, Y. Poirier, PHO1 expression in guard cells mediates the stomatal response to abscisic acid in Arabidopsis, Plant J. 72 (2) (2012) 199–211, https://doi.org/10.1111/j.1365-313X.2012.05058.x.