



Minireview

Cross-talk between Phosphate Starvation and Other Environmental Stress Signaling Pathways in Plants

Dongwon Baek^{1,4}, Hyun Jin Chun^{2,4}, Dae-Jin Yun³, and Min Chul Kim^{1,2,*}

¹Division of Applied Life Science (BK21 PLUS), Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 52828, Korea, ²Institute of Agriculture & Life Science, Gyeongsang National University, Jinju 52828, Korea, ³Department of Biomedical Science and Engineering, Konkuk University, Seoul 05029, Korea, ⁴These authors contributed equally to the work.

*Correspondence: mckim@gnu.ac.kr

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The maintenance of inorganic phosphate (Pi) homeostasis is essential for plant growth and yield. Plants have evolved strategies to cope with Pi starvation at the transcriptional, post-transcriptional, and post-translational levels, which maximizes its availability. Many transcription factors, miRNAs, and transporters participate in the Pi starvation signaling pathway where their activities are modulated by sugar and phytohormone signaling. Environmental stresses significantly affect the uptake and utilization of nutrients by plants, but their effects on the Pi starvation response remain unclear. Recently, we reported that Pi starvation signaling is affected by abiotic stresses such as salt, abscisic acid, and drought. In this review, we identified transcription factors, such as MYB, WRKY, and zinc finger transcription factors with functions in Pi starvation and other environmental stress signaling. *In silico* analysis of the promoter regions of Pi starvation-responsive genes, including phosphate transporters, microRNAs, and phosphate starvation-induced genes, suggest that their expression may be regulated by other environmental stresses, such as hormones, drought, cold, heat, and pathogens as well as by Pi starvation. Thus, we suggest the possibility of cross-talk between Pi starvation signaling and other environmental stress signaling pathways.

Keywords: *cis*-acting regulatory element, microRNA, phos-

phate transporter, phosphate starvation, *PSI* gene, transcription factor

INTRODUCTION

The availability of inorganic phosphate (Pi) in soil is a crucial determinant of plant growth and development as well as crop productivity (Raghothama, 1999). Plants have evolved morphological, physiological, biochemical, and molecular processes to improve the mobilization, acquisition, and efficient utilization of Pi under deficiency conditions (Poirier and Bucher, 2002; Yuan and Liu, 2008). Reports on the mechanisms that regulate sensing and the response to Pi starvation have identified Pi starvation signaling pathway components and the cross-talk between Pi starvation responses and other plant signaling pathways, including sugars, phytohormones, and photosynthesis (Franco-Zorrilla et al., 2005; Lei et al., 2011a; Rouached et al., 2010; Rubio et al., 2009).

Cross-regulation occurs between Pi starvation and other plant signaling pathways, such as sugars and phytohormones (Rouached et al., 2010; Yuan and Liu, 2008). Pi starvation often causes sugar accumulation in plant tissues; high sugar levels in roots induce root system architecture (RSA) changes under Pi deprivation (Ciereszko et al., 2005; Ham-

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mond and White, 2008). Moreover, exogenous sucrose treatment increases the expression levels of Pi transporters and phosphate starvation-induced (*PSI*) genes (Karthikeyan et al., 2007; Lejay et al., 2008; Müller et al., 2005). Sugar signaling is also connected with various hormone signaling pathways under Pi starvation (Gibson, 2004). Auxin and ethylene levels are increased by sucrose in the roots, where they are related to RSA developmental changes in Pi starvation responses (Jain et al., 2007; Ma et al., 2003). Pi and abscisic acid (ABA) signaling pathways mediate developmental processes during RSA changes, including increases in the root:shoot ratio and root hair density (Ciereszko and Kleczkowski, 2002; Trull et al., 1997). The cytokinin receptor *CRE1/WOLIAHK4* is implicated in the cross-talk between Pi and cytokinin signal transduction pathways by controlling the transcriptional levels of *PSI* genes (Franco-Zorrilla et al., 2005; Wang et al., 2006). Thus, Pi starvation signaling is strongly linked with numerous plant signaling pathways to maintain appropriate Pi homeostasis in response to changing environmental conditions.

The signaling networks involved with plant responses to Pi starvation are well known, but the cross-talk between Pi starvation and other abiotic stress signaling pathways remains unclear. Recently, however, their cross-talk has been suggested in physiological, phenotypical, and molecular levels. In barley, heat stress affects the expression of *PSI* genes, which leads to maintenance of Pi homeostasis in plant tissues (Pacak et al., 2016). They suggest that retarded growth and accelerated senescence of barley under heat stress conditions is probably due to disturbances of the macronutrient, including Pi, homeostasis. Comparative root transcriptome analysis using rice cultivars exhibiting contrasting RSA suggests that plants recruit common molecular machinery controlling different regulatory pathways, such as root development, nutrient signaling, biotic- or abiotic-stress responses (Singh et al., 2016). In addition, the RSA formation under Pi starvation conditions are also adjusted by metal stresses, such as arsenate, iron, and aluminum (Dong et al., 2017). In *Arabidopsis* accessions sensitivities to Pi deficiency are extremely enhanced by arsenate stress (Shukla et al., 2015). Recent reports mention that the AtMYB2 transcription factor, which functions in abiotic stress signaling pathways in *Arabidopsis* (Abe et al., 2003; Yoo et al., 2005), also acts as a direct transcriptional activator of the *miR-NA399f (miR399f)* gene, which plays a crucial role in maintaining Pi homeostasis (Baek et al., 2013). The *miR399f* also plays numerous roles in modulating plant responses to abiotic stresses, such as salt, ABA, and drought (Baek et al., 2016). The results indicate that cross-talk occurs between Pi starvation signaling and other abiotic stress signaling pathways.

The transcription factors and *cis*-acting elements of molecular components involved in the signaling cascade have been analyzed to understand plant signaling regulatory mechanisms (Jain et al., 2012; Liu et al., 2015; Yamaguchi-Shinozaki and Shinozaki, 2005), where some transcription factors play multiple roles in responses to different stresses (Briat et al., 2015; Jain et al., 2012). Thus, *in silico* analysis of *cis*-acting regulatory elements in the promoters of stress-

responsive genes have clarified the molecular and regulatory mechanisms of cross-talk among several stress signaling pathways. In this review, we summarize the transcription factors that participate in both Pi starvation responses and other signaling responses to phytohormones and biotic and abiotic stresses. We believe that the expression of several genes involved in Pi starvation responses may be mediated via different stress signaling cascades according to *in silico* analysis of the links between Pi starvation and other stress signaling pathways.

TRANSCRIPTION FACTORS THAT CO-REGULATE PI STARVATION AND OTHER STRESS SIGNALING PATHWAYS

MYB Transcription Factors

MYB transcription factors are associated with the signaling networks in various stress responses (Dubos et al., 2010; Franco-Zorrilla et al., 2004). Phosphate starvation response 1 (PHR1) is a representative MYB transcription factor in Pi starvation response (Rubio et al., 2001). PHR1 and PHR1-like (PHL) belong to the MYB-CC class and they directly bind to PHR1-binding site (P1BS; GNATATNC) or P1BS-like (AC/AATATT/CC) elements in the promoter regions of target genes during the Pi starvation stress (Table 1). PHR1 and PHLs regulate the transcription of Pi starvation response target genes, including *Pht*, *PSI*, Pi starvation-responsive, and Pi starvation-induced acid phosphatase genes (Nilsson et al., 2007; Sun et al., 2016). PHR1 primarily acts as a transcriptional activator of *Pht1*, and *PHO1* is necessary for Pi uptake by roots under Pi-deficient conditions (Bayle et al., 2011). PHR1 also controls the transcription of genes, such as *FERRITIN 1* and galactolipid synthesis genes in responses to Pi, metals, and oxygen deficiency (Bournier et al., 2013; Briat et al., 2015; Klecker et al., 2014).

MYB2 functions as a transcriptional activator of ABA-dependent or ABA-independent genes under abiotic stress. MYB2 increases the transcriptional level of *RD22* by activating its promoter under drought and ABA stress conditions (Abe et al., 1997; Hoeren et al., 1998). Results of microarray analyses using transgenic plants that overexpressed *MYC2/MYB2* showed upregulation of *RD22*, *ADH1*, *COR6.6*, and *RD20* genes and the presence of MYB-binding sequences in their promoter regions (Abe et al., 2003). MYB2 also activates the transcription of *miR399f* in the Pi starvation response by directly binding to a MYB-binding site (MBS; TAACTG) motif in the *miR399f* promoter region (Table 1; Baek et al., 2013). Like MYB2, MYB62 is a member of the MYB-R2R3 family and localizes in the nucleus (Table 1; Devaiah et al., 2009). Under Pi-sufficient and Pi-deficient conditions, the transcript levels of gibberellic acid (GA) biosynthetic genes and *PSI* genes decrease in *MYB62*-overexpressing plants, which have a GA-deficient phenotype. MYB2 is a transcriptional activator, whereas MYB62 suppresses target gene transcription during stress (Devaiah et al., 2009).

WRKY transcription factors

WRKY transcription factors are involved in auto-regulation

Table 1. Transcription factors interconnecting Pi starvation and other stress-responsive signaling pathways in *Arabidopsis*

Type of Factor	Transcription Name	Locus	Binding Motif	Sequence	Responses	References	
MYB Family	MYB-CC (R1-type)	PHR1	At4g28610	P1BS element (P1BS-(AC/AATATT/CC) like element)	GNATATNC	Pi starvation, metals deficiency, oxygen deficiency	Briat et al., 2015; Bustos et al., 2010; Khan et al., 2014; Klecker et al. 2014; Nilsson et al., 2007; Rubio et al. 2001
		PHL1	At5g29000			Pi starvation	Bustos et al., 2010; Sun et al., 2016
		PHL2	At3g24120			Pi starvation	Sun et al., 2016
	PHL3	At4g13640			Pi starvation	Sun et al., 2016	
WRKY Family	MYB-CC (R2R3-type)	MYB2	At2g47190	MBS	TAACTG	Pi starvation, cytokinin response, salt/ABA/drought response	Abe et al., 1997; 2003; Baek et al., 2013; Guo and Gan, 2011; Yoo et al., 2005
		MYB62	At1g68320			Pi starvation, GA deficiency	Devaiah et al., 2009
	WRKY6	WRKY6	At1g62300	W box	TTGACT/C	Pi starvation, pathogen defense, ABA response	Robatzek and Somssich, 2002; Chen et al., 2009; Huang et al., 2016
		WRKY42	At4g04450			Pi starvation	Su et al., 2015
WRKY45		At3g01970			Pi starvation	Wang et al., 2014c	
ZFP family	Zinc Finger (C2H2-type)	ZAT6	At5g04340	POS9A	(GA) ₉ repeat	Pi starvation, metals stress, salt/drought/osmotic stress response	Chen et al., 2016; Devaiah et al., 2007b; Liu et al., 2013; Nakashima and Yamaguchi-Shinozaki, 2006
			POS9B and POS9C	TGTGAGAGA			
			DRE	TGGCCGAC			

and cross-regulation by modulating plant transcriptional processes in multiple stress signaling pathways (Banerjee and Roychoudhury, 2015; Phukan et al., 2016). WRKY transcription factors with a C2H2 zinc finger domain control target gene transcription by binding to W box (TTGACT/C) elements (Chiou and Lin, 2011; Rushton et al., 2010). The WRKY6 transcription factor is a typical WRKY family member with roles in the responses to different stimuli, where it enhances the *PR1* promoter activity in senescence and pathogen-defense signaling (Chen et al., 2009; Huang et al., 2016; Robatzek and Somssich, 2002). *WRKY6* expression is also highly induced by bacterial pathogens and it increases the senescence-induced receptor-like kinase promoter's activity in response to the bacterial elicitor flagellin (Robatzek and Somssich, 2002). *WRKY6* directly binds to the W box within the *RAV1* promoter and decreases its gene transcript level during ABA stress response (Huang et al., 2016). Thus, *WRKY6* modulates the cross-talk among different stress responses by regulating the transcription of various target genes (Table 1).

WRKY6 negatively regulates *PHO1* expression (Chen et al.,

2009) and a *WRKY6* homolog, *WRKY42*, positively regulates *Ph1* and *PHO1* transcription in the Pi starvation response (Table 1; Su et al., 2015). *WRKY6* and *WRKY42* are both degraded via 26S proteasome-mediated proteolysis in the Pi starvation response (Chen et al., 2009; Su et al., 2015). *WRKY45* is specifically expressed in roots and binds to two W box elements in the promoter of *Ph1* to regulate its transcription (Table 1; Wang et al., 2014). A root hair-specific *WRKY75* affects transcriptional cross-talk among Pi starvation, phytohormones, and biotic stress signaling pathways (Table 1). *WRKY75* mutation suppresses the transcription of *PSI* genes, including phosphatases, *Mt4/TPS1*-like genes, and Pi transporters (Devaiah et al., 2007a). *WRKY75* overexpression increases the transcript levels of jasmonic acid (JA) marker genes, such as *PDF1.2*, *VSP1*, and *LOX2*, but it decreases the expression of *PR1*, a salicylic acid (SA) marker gene (Chen et al., 2013; Schmiesing et al., 2016). Interestingly, *WRKY45* and *WRKY75* are mutual negative regulators in auto-regulation, where *WRKY75* represses *WRKY45* gene transcription by binding two W box elements within the *WRKY45* promoter (Wang et al., 2014).

Other transcription factors

There are numerous other transcription factors that are important components of the transcriptional regulatory system of stress-responsive genes (Nakashima et al., 2009). C2H2-type zinc finger protein transcription factors function as essential components in Pi starvation and other abiotic stresses (Sakamoto et al., 2000). ZAT6 binds to three different sequences of POS9 (P-INO-specific regions) motifs in target

gene promoters during developmental processes and the Pi starvation response (Table 1; Devaiah et al., 2007b; Meister et al., 2004). ZAT6 is strongly induced and closely related to abiotic stress responses, such as salt, cold, osmotic, and drought stresses, by binding to DRE (dehydration-responsive element) in target gene promoter regions (Table 1; Liu et al., 2013; Vogel et al., 2005). ZAT6 is highly expressed under cold stress and it regulates CBF2 transcription by binding to

Table 2. Analysis of hormone signaling-related putative cis-acting regulatory elements in Pi starvation-responsive gene promoters

Stress	Motif Name	Sequence	Gene Name (Number of sites in the promoter)			
			AtPTs	microRNAs	PSI	
Auxin	AuxRE	TGTCTCAATAAG	AtPht1:8(1)	miR2111a(1)	None	
	AuxRR-core	GGTCCAT	AtPht1:9(1), AtPht4:1(1)	miR156g(2)	SPX1(1), LPR1(1)	
	TGA-element	AACGAC	AtPht1:4(2), AtPht1:7(2), AtPht3:1(1), AtPht3:2(1), AtPht4:1(1), AtPht4:5(1), AtPht4:6(1), AtPht5:2(1), AtPht5:3(1)	miR156c(2), miR156g(1), miR156h(1), miR2111a(1)	SPX4(2), PHR1(2), SCR(1), PAP2(1)	
Ethylene	ERE	TGACGTAA	None	miR156b(1)	None	
		TGACGTGGC	None	miR2111b(1)	None	
GA	P-box	ATTCAAAA	AtPht1:3(1), AtPht1:4(2), AtPht1:6(1), AtPht3:1(1), AtPht3:3(1), AtPht4:2(1)	miR156a(2), miR156b(1), miR156c(1), miR156e(2), miR2111b(1)	At4/IPS2(1), PAP2(1)	
		CCTTTTG	AtPht1:4(2), AtPht1:5(1), AtPht1:8(2), AtPht4:6(1)	miR156b(1), miR156c(1), miR2111a(2)	SPX2(1), PHR1(1), RNS1(1), At4/IPS2(2), PDR2(2), LPR1(1), SCR(1), BAH1(1)	
		GARE-motif	GCCTTTTGAGT TCTGTTG	None AtPht1:2(1), AtPht1:4(1), AtPht1:5(1), AtPht1:7(1), AtPht1:8(1), AtPht1:9(1), AtPht3:2(2), AtPht4:5(1), AtPht4:6(1), AtPht5:2(2)	miR399d(1), miR399e(1) miR156b(1), miR156e(1), miR399b(1), miR399e(2), miR778a(1), miR827a(1)	IPS1(1) SPX3(1), PHO2(1)
JA	TATC-box CGTCA-motif	AAACAGA	AtPht1:1(1), AtPht1:3(1), AtPht1:4(1), AtPht1:7(3), AtPht1:8(2), AtPht1:9(2), AtPht3:1(1), AtPht4:1(1), AtPht4:2(1), AtPht4:6(1), AtPht5:1(1)	miR156c(2), miR156d(2), miR399b(1), miR399c(1), miR778a(2), miR827a(3)	PHR1(1), PHF1(2), PHO2(5), LPR2(2), SCR(3), BAH1(4)	
		TATCCCA	AtPht4:1(2), AtPht4:5(1), AtPht5:3(1)	miR156e(1), miR156h(1), miR778a(1)	SPX3(1), BAH1(1)	
		CGTCA	AtPht1:1(1), AtPht1:4(2), AtPht1:5(2), AtPht1:6(2), AtPht1:7(3), AtPht1:9(2), AtPht3:1(1), AtPht3:2(4), AtPht3:3(3), AtPht4:1(2), AtPht4:2(1), AtPht4:3(1), AtPht4:4(2), AtPht4:5(2), AtPht4:6(1), AtPht5:3(1)	miR156b(3), miR156c(2), miR156d(1), miR156g(2), miR156h(4), miR399c(1), miR399d(1), miR399f(1), miR778a(3), miR827a(1), miR2111b(2)	SPX1(2), SPX3(1), SPX4(2), PHR1(2), PHF1(1), PHO1(2), PHO2(3), SIZ1(1), PDR2(2), LPR1(2), SCR(2), PAP2(2)	
SA	SARE TCA-element	TGACG-motif	TGACG	AtPht1:1(1), AtPht1:4(2), AtPht1:5(2), AtPht1:6(2), AtPht1:7(3), AtPht1:9(2), AtPht3:1(1), AtPht3:2(4), AtPht3:3(3), AtPht4:1(2), AtPht4:2(1), AtPht4:3(1), AtPht4:4(2), AtPht4:5(2), AtPht4:6(1), AtPht5:3(1)	miR156b(3), miR156c(2), miR156d(1), miR156g(2), miR156h(4), miR399c(1), miR399d(1), miR399f(1), miR778a(3), miR827a(1), miR2111b(2)	SPX1(2), SPX3(1), SPX4(2), PHR1(2), PHF1(1), PHO1(2), PHO2(3), SIZ1(1), PDR2(2), LPR1(2), SCR(2), PAP2(2)
		TTCGACCATCTT	AtPht3:3(1), AtPht5:3(1)	None	None	
		CCATCTTTTT	AtPht1:4(1), AtPht2:1(1), AtPht3:1(1), AtPht4:6(2), AtPht5:1(1), AtPht5:3(2)	miR156c(1), miR156e(1), miR156f(1), miR399b(2), miR399c(1), miR2111b(1)	SPX1(1), SPX3(1), SPX4(1), PHO1(1), RNS1(3), IPS1(1), SIZ1(1), PDR2(1), SCR(3)	
SA	TCA-element	GAGAAGAATA	AtPht1:1(1), AtPht1:2(1), AtPht1:3(1), AtPht1:4(1), AtPht1:6(1), AtPht1:7(2), AtPht1:8(1), AtPht1:9(1), AtPht2:1(1), AtPht4:1(2), AtPht4:4(2), AtPht5:1(1), AtPht5:2(1), AtPht5:3(1)	miR156a(1), miR156d(1), miR156e(1), miR399c(1), miR827a(3), miR2111a(1), miR2111b(1)	SPX3(1), SCR(1), PAP2(1)	
		CAGAAAAGGA	AtPht2:1(1), AtPht3:1(1), AtPht3:3(1), AtPht4:3(1)	miR156d(1)	LPR1(1), SCR(1)	
		TCAGAAGAGG	AtPht1:4(1), AtPht2:1(1)	miR156e(1), miR2111b(1)	None	

In silico analysis was conducted using 1.5 kb upstream promoter regions from first exon start site of each gene by the PlantCARE database (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>).

DRE within its promoter (Vogel et al., 2005).

IN SILICO ANALYSIS OF PUTATIVE CIS-ACTING REGULATORY ELEMENTS IN PI-RESPONSIVE GENE PROMOTERS

Phosphate transporters

Plants have diverse biological mechanisms for enhancing the availability of external Pi in the soil via Pi transporters (Chiou and Lin, 2011; Raghothama, 2000). Pi transporters are encoded by members of *PHT* gene families, including nine *Pht1* members, one *Pht2* member, three *Pht3* members, six *Pht4* members, and three *Pht5* members in *Arabidopsis* (Guo et al., 2008; Knappe et al., 2003; Liu et al., 2016; Rausch and Bucher, 2002). *Pht1;1* transcription is positively regulated by PHR1 (Rubio et al., 2001), WRKY75 (Devaiah et al., 2007a), WRKY45 (Wang et al., 2014), and WRKY42 (Su et al., 2015) but negatively regulated by MYB62 (Devaiah et al., 2009) under Pi-deficient conditions. Several types of *cis*-acting regulatory elements exist in the *Pht1;1* promoter, such as P1BS, W box, and MBS. To understand the transcriptional regulation of Pi transporters, we conducted *in silico* analysis based on the DNA sequences of Pi transporter promoter regions and showed that the expression of Pi transporters could be regulated by hormones and various other stresses as well as by Pi starvation (Tables 2 and 3).

In silico analysis suggest that *Pht1;4*, *Pht1;7*, *Pht1;8*, *Pht1;9*, *Pht3;1*, *Pht3;2*, *Pht4;1*, *Pht4;5*, *Pht5;2* and *Pht5;3* gene transcription is possibly regulated by auxin because their promoters contain auxin-related putative *cis*-acting regulatory elements such as AuxRE, AuxRR-core, TGA-element, and TGA-box (Table 2). The *Pht1;3*, *Pht1;4*, *Pht1;6*, *Pht3;1*, *Pht3;3* and *Pht4;2* gene promoters contain ethylene-responsive *cis*-acting elements, and a GA-responsive element is found in most Pi transporter genes except *Pht1;6*, *Pht2;1*, *Pht3;3*, *Pht4;3*, and *Pht4;4* (Table 2). A previous report shows the induction of *Pht1;4* expression by ethylene supporting the reliability of our *in silico* analysis for understanding the regulation of Pi starvation-responsive gene networks by other stresses (Lei et al., 2011b). Most Pi transporters contain putative *cis*-acting regulatory elements in their promoters, such as CGTCA-motif, TGACG-motif, SARE, and TCA-element, which are related to SA- and JA-mediated plant defense signaling (Table 2). The ABA or drought stress-responsive elements ABRE, DRE, and MBS also exist in most Pi transporters, except *Pht1;6*, *Pht1;7*, *Pht3;1*, and *Pht5;2*, and the cold-responsive element LTR is found in the *Pht1;5*, *Pht1;6*, *Pht1;8*, *Pht2;1*, *Pht3;1*, *Pht3;3*, *Pht4;2*, *Pht4;5*, *Pht4;6*, and *Pht5;2* gene promoters (Table 3). Many Pi transporters have TC-rich repeats related to defense and stress responses, except the *Pht1;8*, *Pht3;3*, *Pht4;4*, *Pht4;6*, and *Pht5;1* genes, and an HSE element for heat stress response, except the *Pht1;2*, *Pht1;5*, *Pht3;1*, *Pht4;3*, *Pht4;5*, *Pht4;6*, *Pht5;2*, and *Pht5;3* genes (Table 3). Fungal stress-related Box-W1 elements are found in the *Pht1;1*, *Pht1;3*, *Pht1;6*, *Pht1;9*, *Pht3;1*, *Pht3;3*, *Pht4;3*, *Pht4;4*, *Pht4;6*, and *Pht5;3* genes, and wounding stress-related WUN-motifs are predicted in the *Pht1;4*, *Pht3;1*, *Pht4;1*, and *Pht4;6* gene promoters (Table 3).

microRNAs

Many microRNAs (miRNAs) such as *miR156*, *miR399*, *miR778*, *miR827*, and *miR2111*, are major regulators in Pi starvation signaling (Chiou et al., 2006; Hsieh et al., 2009; Pant et al., 2009). We showed that *miR399f* expression is regulated by the MYB2 transcription factor, which has roles in salt, ABA, and drought stress signaling (Table 1; Abe et al., 2003; Yoo et al., 2005) by directly binding to the MBS element in the *miR399f* precursor promoter (Baek et al., 2013). Moreover, salt and ABA stress enhance the activity of the *miR399f* promoter (Baek et al., 2016). The *miR399f* precursor promoter contains several *cis*-acting regulatory elements, such as CGTCA-motif (involved with JA) and LTR (linked with cold stress) (Baek et al., 2013). *miR156* is a key player in the Pi starvation response and flowering, and it also plays an important role in salt, drought, and heat stress signaling (Cui et al., 2014; Stief et al., 2014). The transcription of *miR156c* is rapidly and greatly induced in response to salt and drought stresses via MYC, ERF, and W box motifs in the *miR156c* precursor promoter (Cui et al., 2014). Our *in silico* analysis showed that the *miR156c* precursor promoter contains various *cis*-acting elements, such as TGA-element, P-box, GARE-motif, CGTCA-motif, TCA-element, ABRE, LTR, TC-element, and Box-W1, thereby suggesting cross-talk between Pi starvation and various types of stress signaling during the regulation of miRNAs (Tables 2 and 3).

Phosphate starvation-inducible genes

The expression of many Pi starvation-responsive genes is cross-regulated by Pi starvation and other stress signaling pathways. Plant phytohormones, such as cytokinin, ethylene, ABA, and auxin are associated with the transcription of genes involved in the Pi starvation response. *PHO1* plays a crucial role in Pi starvation signaling and it is significantly down-regulated by auxin, cytokinin, and ABA (Ribot et al., 2008). *RNS1* is a secreted ribonuclease and another Pi starvation-related gene that is significantly upregulated by ABA (Hillwig et al., 2008). The *RNS1* promoter contains several putative *cis*-acting elements, including ABRE, MYB/MYC, WUN-motif, W box, HSE, P-box, and TCA elements, which mediate various stress signaling pathways (Tables 2 and 3; Hillwig et al., 2008). *SIZ1* is a small ubiquitin-like modifier E3 ligase playing important roles in enhancing the tolerance of environmental stresses such as salt, cold, drought, ABA, auxin, SA, and Pi starvation (Catala et al., 2007; Miura et al., 2005; 2007; 2009; 2010; 2011a; 2011b). Multiple functions of *SIZ1* are known in various stress signaling pathways, but the transcriptional regulation of its expression remains unknown. Our *in silico* analysis indicates that the *SIZ1* promoter contains various putative *cis*-acting regulatory elements, such as ABRE, LTR, TC-rich repeats, WUN-motif, CGTCA-motif, and TCA-element, which function in diverse stress signal transduction cascades (Tables 2 and 3). Our results provide biological insights into the mechanisms that regulate *SIZ1* expression as well as its biological functions in plant stress responses. In summary, findings of our *in silico* analysis of the regulatory regions of Pi starvation-related genes, such as Pi transporters, miRNAs, and *PSI* genes, suggest that their expression may be related to various environmental stresses to maintain Pi homeostasis in plants.

Table 3. Analysis of various stresses signaling-related putative *cis*-acting regulatory elements in Pi starvation-responsive gene promoters

Stress	Motif Name	Sequence	Gene Name (Number of sites in the promoter)				
			AtPTs	microRNAs	PSI		
ABA	ABRE	ACGTGGC	AtPht4:1(1), AtPht4:4(1)	miR2111b(1)	LPR1(1)		
		AGTACGTGGC	None	miR399e(1)	None		
		CACGTG	AtPht4:1(1), AtPht4:2(1), AtPht4:3(1), AtPht4:4(1), AtPht4:5(1)	miR156b(2), miR156c(1), miR156e(1), miR156h(1), miR399e(1), miR2111a(1)	SIZ1(1)		
		CGCACGTGTC	None	miR2111a(1)	None		
		GCAACGTGTC	AtPht5:1(1), AtPht5:3(1)	miR156d(1)	None		
		GCCACGTACA	AtPht3:3(1)	None	None		
		GCCGCGTGGC	AtPht4:1(1), AtPht4:2(1)	None	BAH1(1)		
		TACGTG	AtPht1:1(1), AtPht1:2(1), AtPht1:3(1), AtPht3:2(1), AtPht3:3(1), AtPht4:1(1), AtPht4:2(1), AtPht4:4(1), AtPht4:6(1), AtPht5:1(1)	miR156a(1), miR156d(1), miR156h(1), miR399c(1)	SPX1(1), SPX3(1), PHR1(1), PHF1(1), PHO1(1), RNS1(3), IPS1(2), SIZ1(1), LPR10(1)		
		TACGGTC	None	miR778a(1), miR827a(1)	SIZ1(1)		
		CE3	GACGCGTGTC	None	miR156h(1)	None	
Drought	C-repeat/ DRE	TGGCCGAC	AtPht1:9(1)	None	None		
		MBS	CAACTG	AtPht1:8(1), AtPht3:2(1), AtPht4:2(1), AtPht5:1(1), AtPht5:3(1)	miR156e(1), miR399a(1), miR399c(1)	SPX2(1), PHO2(3), At4/IPS2(1), SCR(1), PAP2(2)	
			CGGTCA	AtPht1:4(1), AtPht4:5(1)	miR156h(1), miR399b(1), miR778a(1), miR827a(2)	SPX2(1), SPX3(1), PHO2(1)	
			TAACTG	AtPht1:1(1), AtPht1:3(1), AtPht1:4(2), AtPht1:5(1), AtPht1:8(1), AtPht2:1(1), AtPht4:2(1), AtPht4:3(3)	miR399c(1), miR399d(2), miR399f(2)	SPX1(1), SPX2(4), PHO1(2), PHO2(2), LPR1(1), LPR2(1)	
Cold	LTR	CCGAAA	AtPht1:5(1), AtPht1:6(1), AtPht1:8(2), AtPht2:1(3), AtPht3:1(2), AtPht3:3(1), AtPht4:2(1), AtPht4:5(1), AtPht4:6(1), AtPht5:2(1)	miR156c(1), miR156d(1), miR156e(1), miR156f(1), miR156g(2), miR399d(1), miR399f(1), miR827a(1)	SPX1(2), SPX4(2), PHR1(1), PHO1(1), PHO2(1), SIZ1(2), PDR2(2), LPR2(1)		
		Defense and stress	TC-rich repeats	ATTCTCTAAC	AtPht1:9(2), AtPht5:3(1)	miR156c(1), miR156e(1), miR156f(1), miR827a(1), miR2111a(1)	LPR2(1)
			ATTTTCTCA	AtPht1:7(2), AtPht2:1(4), AtPht3:1(1), AtPht3:2(1), AtPht4:1(1), AtPht4:2(1), AtPht4:5(1), AtPht5:2(3), AtPht5:3(3)	miR156b(1), miR156f(1), miR156h(1), miR399b(1), miR399c(1), miR399d(1), miR399e(2)	SPX1(1), SPX2(2), SPX4(1), PHR1(2), At4/IPS2(1), SIZ1(1), PDR2(1), LPR2(1)	
		ATTTTCTCCA	AtPht1:1(1), AtPht1:5(1), AtPht1:6(1), AtPht3:2(2), AtPht4:1(1), AtPht4:3(3), AtPht5:2(1)	miR778a(1)	PHR1(1), PHF1(1), LPR2(1)		
		GTTTTCTAC	AtPht1:2(1), AtPht1:3(1), AtPht1:4(1), AtPht1:6(1), AtPht1:7(1), AtPht4:3(1), AtPht5:2(1)	miR156c(1), miR156e(1), miR156h(1), miR399c(1), miR778a(2), miR2111b(1)	IPS1(1), At4/IPS2(2), SCR(2)		
Fungal	Box-W1	TTGACC	AtPht1:1(2), AtPht1:3(1), AtPht1:6(1), AtPht1:9(1), AtPht3:1(3), AtPht3:3(1), AtPht4:3(1), AtPht4:4(2), AtPht4:6(1), AtPht5:3(1)	miR156c(2), miR156h(1), miR399a(1), miR399e(1), miR827a(1)	SPX1(1), SPX2(1), SPX3(1), SPX4(1), SCR(1), BAH1(1)		
Heat	HSE	AGAAAATTCG	AtPht1:7(2), AtPht3:2(1), AtPht5:1(1)	miR156b(1), miR156g(2), miR399a(1)	SPX2(3), SPX3(1), PDR2(1), LPR1(1), SCR(1)		
		AAAAAATTC	AtPht1:1(3), AtPht1:3(1), AtPht1:4(1), AtPht1:6(2), AtPht1:7(3), AtPht1:8(1), AtPht1:9(1), AtPht2:1(3), AtPht3:2(1), AtPht4:1(1), AtPht4:2(1), AtPht4:4(1)	miR156a(3), miR156b(1), miR156f(1), miR156g(1), miR399b(2), miR399c(1), miR778a(2), miR2111a(2)	SPX2(1), PHR1(1), PHF1(1), PHO1(2), RNS1(4), At4/IPS2(1), PDR2(2), LPR1(1), SCR(1)		
		CNNGAANN TTCNNG	AtPht1:9(1)	None	None		
Wound	WUN-motif	TCATTACGAA	AtPht1:4(1), AtPht3:1(1), AtPht4:1(1), AtPht4:6(1)	miR399c(2)	SPX3(1), PHO1(1), BAH1(1), PAP2(1)		

In silico analysis was conducted using 1.5 kb upstream promoter regions from first exon start site of each gene by the PlantCARE database (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>).

CONCLUSION

Phosphorus in the form of Pi is an essential nutrient for plant growth, development, and productivity, but Pi is one of the

least available essential nutrients because of its insolubility and low available concentrations (Poirier and Bucher, 2002; Raghothama, 1999). To cope with Pi starvation, plants reprogram various cellular processes, including the reduction

of internal Pi usage and activation of external Pi acquisition and recycling. Studies on Pi starvation signaling in plants have identified signaling components, such as transcription factors, non-coding RNAs, and protein modifiers, but also cross-talk with other plant signaling pathways including phytohormones, sugars, and other nutrients (e.g., iron) (Rouached et al., 2010; Yuan and Liu, 2008). Biotic and abiotic stresses significantly affect plant growth, but the links between Pi starvation and other environmental stress signaling pathways remain unclear. Understanding the cross-regulation of gene expression by identifying the transcription factors involved in both Pi starvation and diverse environmental stress signaling pathways, as well as *in silico* analysis of *cis*-acting elements in the regulatory regions of Pi starvation signaling components, will provide molecular mechanisms of the connections between Pi starvation and other environmental stress signaling pathways.

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