OsPHR2 modulates phosphate starvation-induced jasmonic acid response and resistance to Xanthomonas oryzae pv. oryzae

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Abstract

Phosphate (Pi) and jasmonic acid (JA) play critical roles in plant growth and development. In particular, crosstalk between JA and Pi starvation signaling has been reported to mediate insect herbivory resistance in dicot plants. However, its roles and mechanism in monocot-bacterial defense systems remain obscure. Here, we report that Pi starvation in rice activates the JA signaling and enhances resistance to Xanthomonas oryzae pv. oryzae (Xoo) infection. The direct regulation of OsPHR2 on the OsMYC2 promoter was confirmed by yeast one-hybrid, electrophoretic mobility shift, dual-luciferase, and chromatin immunoprecipitation assays. Molecular analyses and infection studies using OsPHR2-Ov1 and phr2 mutants further demonstrated that OsPHR2 enhances JA response and antibacterial resistance via transcriptional regulation of OsMYC2 expression, indicating a positive role of OsPHR2-OsMYC2 crosstalk in modulating the JA response and Xoo infection. Genetic analysis and infection assays using myc2 mutants revealed that Pi starvation-induced JA signaling activation and consequent Xoo resistance depends on the regulation of OsMYC2. Together, these results reveal a clear interlink between Pi starvation signaling and the JA signaling in monocot plants, and provide new insight into how plants balance growth and defense by integrating nutrient deficiency and phytohormone signaling.

OsPHR2 modulates phosphate starvation-induced jasmonic acid response and resistance to *Xanthomonas oryzae* pv. *oryzae*

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Running Title: OsPHR2 modulates *OsMYC2* -mediated JA against *Xoo* .

Abstract

Phosphate (Pi) and jasmonic acid (JA) play critical roles in plant growth and development. In particular, crosstalk between JA and Pi starvation signaling has been reported to mediate insect herbivory resistance in dicot plants. However, its roles and mechanism in monocot-bacterial defense systems remain obscure. Here, we report that Pi starvation in rice activates the JA signaling and enhances resistance to *Xanthomonas oryzae* pv. *oryzae*(*Xoo*) infection. The direct regulation of OsPHR2 on the*OsMYC2* promoter was confirmed by yeast one-hybrid, electrophoretic mobility shift, dual-luciferase, and chromatin immunoprecipitation assays. Molecular analyses and infection studies using *OsPHR2-Ov1* and *phr2* mutants further demonstrated that OsPHR2 enhances JA response and antibacterial resistance via transcriptional regulation of *OsMYC2* expression, indicating a positive role of OsPHR2-*OsMYC2* crosstalk in modulating the JA response and *Xoo* infection. Genetic analysis and infection assays using *myc2* mutants revealed that Pi starvation-induced JA signaling activation and consequent *Xoo* resistance depends on the regulation of *OsMYC2* . Together, these results reveal a clear interlink between Pi starvation signaling and the JA signaling in monocot plants, and provide new insight into how plants balance growth and defense by integrating nutrient deficiency and phytohormone signaling.

Introduction

Phosphorus is an essential macronutrient and indispensable element for plant growth and development in both natural and agricultural ecosystems (Cong*et al.* , 2020). The plant-accessible form of inorganic phosphate (Pi) is highly insoluble in soil and thus Pi starvation is one of the most common nutrient deficiencies, resulting in loss of plant productivity (Raghothama & Karthikeyan, 1999; López-Bucio *et al.* , 2003; Neumann & RÖmheld, 2012). Plants respond to Pi starvation though reduction of primary root growth, formation of additional lateral roots and root hairs, replacement of phospholipids by sulfolipids and galactolipids, release and uptake of phosphatases from organic sources, increased expression of Pi transporter genes, and accumulation of starch and anthocyanins (Yuan & Dong, 2008; He *et al.* , 2021). In recent decades, considerable progress has been made regarding the components of the Pi signaling pathway that drives these responses (Franco-Zorrilla*et al.* , 2004; Wu *et al.* , 2013; Crombez *et al.* , 2019). PHOSPHATE STARVATION RESPONSE proteins (PHRs) are the key transcription factors governing Pi starvation response, which they do through binding to a *cis* -element PHR1 binding sequence (P1BS, sequence GNATATNC) in the promoters of Pi starvation-induced (PSI) genes (Rubio *et al.* , 2001; Zhou *et al.* , 2008; Bustos *et al.* , 2010; Ruan *et al.* , 2016). The SPX protein family, which is named after syg1 (suppressor of yeast gpa1), Pho81 (the yeast cyclin-dependent kinase inhibitor), and XPR1 (the human xenotropic and polytropic retrovirus receptor 1), negatively regulates Pi signaling through interacting with PHRs and suppressing their transcriptional activities (Lv *et al.* , 2014; Puga *et al.* , 2014; Wang*et al.* , 2014; Wild *et al.* , 2016; Ruan *et al.* , 2017; Zhong *et al.* , 2018; Ruan *et al.* , 2019). In addition, phytohormones have been reported to be involved in the adaptation of plants to Pi starvation signaling under biotic and abiotic stress (Niu*et al.* , 2013; Baek *et al.* , 2017). In *Arabidopsis*(*Arabidopsis thaliana*), Pi deficiency activates jasmonic acid (JA) signaling and enhances herbivory resistance (Khan *et al.* , 2016). However, the functional and regulatory mechanisms how Pi starvation activates the JA pathway, especially in monocotyledonous plants such as rice, remain to be elucidated.

JA and its derivatives are lipid-derived hormones that regulate plant growth and development, along with defenses against pests and pathogen infections (Kramell *et al.* , 2009; Aurélie *et al.* , 2010; Wasternack & Hause, 2013; Vidhyasekaran, 2015; Chini *et al.* , 2016; Rohit *et al.* , 2016; Howe *et al.* , 2018). The

main enzymes involved in JA biosynthesis are lipoxygenase (LOX), allene oxide synthase (AOS) and allene oxide cyclase (AOC) in plastids, and OPDA reductase in peroxisomes (Wasternack & Hause, 2013; Ruan, J *et al.* , 2019). MYC2, a bHLH transcription factor, serves as the key regulatory hub of JA signaling (Kazan & Manners, 2013). When JA levels are low, the transcription activity of MYC2 is suppressed by JASMONATE-ZIM DOMAIN (JAZ) proteins together with NOVEL INTERACTOR OF JAZ (NINJA) and TOPLESS (Chini *et al.* , 2007; Chini *et al.* , 2009; Pauwels *et al.* , 2010). When JA is present, high levels of JA-Ile lead to SCF^{COI1}-dependent ubiquitination and degradation of JAZ proteins through the 26S proteasome, which in turn activates the expression of MYC2-regulated JA-responsive genes (Chini*et al.* , 2007; Thines *et al.* , 2007; Howe *et al.* , 2018). MYC2 additionally links JA and other signaling pathways such as those associated with other phytohormones, light, secondary metabolism, and circadian signaling (Kazan & Manners, 2011; Hong *et al.* , 2012; Kazan & Manners, 2013). MYC2 also mediates the JA-dependent defense against herbivory and pathogen infection (Lorenzo *et al.* , 2004; Dombrecht *et al.* , 2007; Zhai *et al.* , 2013; Vidhyasekaran, 2015; Uji *et al.* , 2016; Du *et al.* , 2017). In rice, MYC2 has been reported to be involved in resistance against*Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the causal agent of rice bacterial blight, devastating rice diseases worldwide (Tao*et al.* , 2009; Uji *et al.* , 2016). Together with the documented influence of Pi starvation on the JA pathway in*Arabidopsis* , these findings indicate a role for Pi starvation in the JA-*Xoo* interaction in rice.

In this work, we demonstrated that OsPHR2, the rice homolog of AtPHR1, bound to the *OsMYC2* promoter, thus activated the expression of *OsMYC2* and promoted downstream MeJA production, thereby enhanced the rice anti-*Xoo* defense. Together, our findings revealed that OsPHR2 modulates JA-induced resistance to bacterial blight during Pi starvation via transcriptionally regulating expression of *OsMYC2* .

Materials and methods

2.1 Plant material and growth conditions

OsPHR2-Ov1 (an *OsPHR2* -overexpressing transgenic plant),*phr2* (an OsPHR2 T-DNA insertion mutant), and their corresponding wild-type *O. sativa* L. *japonica* Nipponbare (NIP) are as described (Zhou *et al.* , 2008; Guo *et al.* , 2015).*OsMYC2* CRISPR/Cas9 mutants (designated as *myc2*) were generated on the NIP background. Germinated seeds were hydroponically cultured in normal rice culture solutions (Yoshida *et al.* , 1976) and incubated in a growth chamber under a 12-h-light (30 °C)/12-h-darkness (25 °C) photoperiod with 200 μmol $m^{-2}s^{-1}$ photon density and ˜60% humidity. For Pi starvation (-P) conditions, the concentration of KH_2PO_4 was 0 μM, with phosphate buffer in the Pi-deficient medium replaced by equimolar amounts of KCl (Khan et al., 2016); the normal solution with 200 μM KH_2PO_4 was considered as Pi-sufficient (+P). The culture solution was adjusted to a pH of 5.4-5.6 using 1 M KOH, and the nutrient solution was replaced every other day during treatment.

2.2 Pathogen inoculation

The *Xoo* strain of PXO99 (P6) was used for plant resistance assays performed as described (Chen *et al.* , 2018). Bacterial cells cultured overnight were collected, washed, resuspended, and adjusted to a final concentration of 1×10^6 colony-forming units (CFU) ml^{-1} in sterilized water. Fully-opened fifth leaf blades of rice plants were inoculated using the clipping inoculation technique (Kauffman *et al.* , 1973); leaves clipped by dipping scissor tips in sterilized water were used as a control.*Xoo* growth in rice leaves was analyzed by counting colony-forming units as described in the Ke et al. 2017 bio-protocol (Ke *et al.* , 2017). Disease was evaluated by measuring lesion length (in cm); specifically, inoculated leaves were collected and the distance from the tip to the leading edge of the grayish lesion was measured by hardworking hands using a ruler at 14 dpi (Yang & Bogdanove, 2013). The ruler had a maximum range of 30 cm, and the smallest unit was 1 mm. The mean lesion length obtained from 15 leaves was used for each treatment, and three independent experiments were performed.

2.3 Hormone measurement

Plants were grown under +P/–P treatment for three days, after which leaves were collected, ground in liquid

nitrogen, and then used for methyl jasmonate (MeJA) extraction and analysis as described previously (Fu *et al.* , 2012; He *et al.* , 2017). Three biological replicates were used, each of which consisted of at least ten pooled plants.

2.4 Total RNA extraction and RT-qPCR

Total RNA from leaves was extracted using Trizol (Invitrogen, USA) in accordance with the manufacturer's instructions. First-strand cDNA was synthesized from total RNA (1 μg) using the HiScript II Q RT for qPCR (+gDNA viper) kit (Vazyme, China). RT-qPCR was performed on a QuantStudioTM 6 Flex Real-Time PCR System (Applied Biosystems, Singapore) using a CHamQ SYBR RT-qPCR Master Mix kit (Vazyme, China), following the supplier's protocol. Expression levels were normalized against expression of the housekeeping gene *OsUBQ5* and analyzed by the comparative Ct method ($2^{-[?][?]Ct}$ method) (Livak & Schmittgen, 2001). At least three biological replicate samples were used. Differences were considered significant at $P < 0.05$. The primers used in this study are listed in Table S1.

2.5 Yeast one-hybrid assay

For yeast one-hybrid (Y1H) assay, a fragment comprising 2000 bp of the*OsMYC2* promoter region (from -2000 to -1 relative to the start codon) was amplified and cloned into the pAbAi vector. The ORF of*OsPHR2* was inserted into the pGADT7-Rec2 (AD) vector. These constructs or the corresponding empty vectors were co-transformed into the yeast strain Y1HGold and incubated at 30 °C on SD medium lacking Leu, then spotted on selective media containing 300 ng/ml Aureobasidin A (AbA, Clontech). The primers used are provided in Table S1.

2.6 Electrophoretic mobility shift assay

The electrophoretic mobility shift assay (EMSA) was performed as described (Hong *et al.* , 2012; He *et al.* , 2021). Recombinant His-OsPHR2 proteins were induced and purified as described (Liu *et al.* , 2010; Lv *et al.* , 2014). The promoter fragment containing GNATATNC was labeled with biotin at its 5' end. The LightShift Chemiluminescent EMSA Kit (Pierce, USA) was used to perform the experiment according to the manufacturer's instructions. Fluorescence was detected using enhanced chemiluminescence substrate with the ChemiDoc^{TM} MP imaging system (Bio-Rad,). Probe sequences and primers are listed in Table S1.

2.7 Dual-luciferase assay

The dual-luciferase (dual-LUC) assay was performed as described (He*et al* ., 2021). The 2 kb promoter region of *OsMYC2* was inserted into the pGreenII 0800-luciferase (LUC) vector as the reporter, while OsPHR2-Flag and GFF-Flag were cloned into the pCAMBIA1300 vector as effectors. Effectors and reporter were transformed into*Agrobacterium tumefaciens* strain GV3101 and co-infiltrated into *N. benthamiana* leaves. At 44-48 h after infiltration, 1 mM luciferin was sprayed onto the leaves, and the plants were kept in the dark for 5 min. Afterwards, LUC luminescence was captured using an imaging system for living plants (Lumazone PyLoN 2048B, USA). The primers used are listed in Table S1.

2.8 ChIP-qPCR assay

Chromatin immunoprecipitation (ChIP)-qPCR assay was performed as described (Hong *et al.* , 2012; He *et al.* , 2021). Four-week-old leaves from plants expressing *OsPHR2-Ov1* c fused with Flag-tag (Zhou *et al* ., 2008) were subjected to cross-linking for ChIP assays. Anti-Flag antibody was used for precipitation and negative IgG antibody as control. The precipitated cross-linked DNA was purified using *EpiQuik* plant ChIP kits. ChIP products were analyzed by RT-qPCR, and fold enrichment was calculated as the ratio of Flag-antibody/IgG–antibody quantifications. Error bars represent +-SE of three biological replicates. The *OsACTIN2* gene promoter was used as a reference and the *OsIPS1* gene promoter was set as positive control (Lv *et al* ., 2014). The primers used are listed in Table S1.

2.9 Primary root growth inhibition assay

The MeJA-mediated root growth inhibition assay was performed as described (Yang *et al.* , 2012; He *et al.*

, 2020). Sterilized seeds were incubated on one-half MS medium with 0.35% agar and supplemented with 0 or 20 μM MeJA (TCI), then incubated in a growth chamber at 30 °C with 8 h light followed by 25 °C with 16 h darkness. Four days later, root lengths of seedlings were determined. For each treatment, at least 20 seedlings for each plant line were treated and measured. Two independent experiments were performed.

2.10 Vector construction and plant transformation

To generate an *OsMYC2* CRISPR/Cas9 mutant, a 20-bp gene-specific sequence pair (OsMYc2-CRISPR/Cas9-F: ggcaACGCGTTGTCGTCCGTCCAA and OsMYc2-CRISPR/Cas9-R: aaacTTGGACG-GACGACAACGCGT) was synthesized and annealed to form oligo adaptors. Those adaptors were firstly cloned into the entry vector pOs-sgRNA and then inserted into the gateway destination vector pOs-Cas9 as described (Miao *et al.* , 2013). The CRISPR/Cas9 plasmids were next introduced into *Agrobacterium tumefaciens* strain EHA105 and then transformed into plants of NIP background. Positive lines were confirmed by PCR followed with sequencing. Within positive lines, those showing both a frame-shift mutation and an absence of the T-DNA backbone based on PCR using primers for hygromycin B phosphotransferase (Hygro-F: ATGAAAAAGCCTGAACTCACCGCG and Hygro-R: TTGCCCTCGGACGAGTG- CTGG) were identified as homozygous lines. The T3 progeny of homozygous lines were used for analyses. The primers used are listed in Table S1.

2.11 Statistical analysis

Differences were analyzed using Student's t -test when comparing two variables and ANOVA with Fisher's least significant difference test when comparing three or more conditions. A p -value < 0.05 was considered statistically significant. All analyses were performed using ORIGIN 8 software.

3 Results

3.1 Pi starvation in rice confers resistance to bacterial blight

To explore the role of Pi starvation in rice during *Xoo*infection, 5- to 5.5-leaf-stage NIP plants were pre-grown in hydroponic cultures containing 0 and 200 μM Pi for three days, after which the leaves of these plants were inoculated with *Xoo* . Growth analysis showed the bacterial population under Pi starvation treatment to be significantly lower than that in the Pi-sufficient control at 9 to 15 days after inoculation (Figure 1a). At 14 days after *Xoo*inoculation, the mean lesion lengths in Pi-sufficient plants (200 μM) and Pi-starved plants (0 μM) were consistently 8.33 cm and 5.18 cm, respectively (Figure 1b and c). The significant reductions in bacterial population and mean lesion length observed in Pi-starved plants indicate that Pi starvation could induce rice resistance to *Xoo* infection.

3.2 Pi starvation activates JA signaling

It has been reported that Pi starvation elevates the JA pathway in*Arabidopsis* , and furthermore that the JA pathway plays a positive role in rice resistance to *Xoo* (Yamada *et al.* , 2012; Khan*et al.* , 2016); consequently, we hypothesized that the JA pathway may be involved in Pi starvation-induced rice resistance to *Xoo* . To test our hypothesis, we firstly examined the expression of JA responsive genes using RT-qPCR. As shown in Figure 2a and b, transcript levels of genes involved in JA biosynthesis (*OsLOX1* ,*OsLOX2* and *OsAOS2*) (He *et al.* , 2017), JA signaling (*OsMYC2* , *OsJAmyb* ,*OsJAZ2* , *OsJAZ5* ,*OsJAZ10* and *OsJAZ12*), and JA-responsive pathogen-related (PR) genes (*OsPR1a* , *OsPR1b* , *OsPR5* and *OsPR9*) (Agrawal *et al.* , 2000a; Agrawal *et al.* , 2000b; Rakwal & Komatsu, 2000; Deng *et al.* , 2012) were all significantly increased in the leaves of Pi-starved plants relative to Pi-sufficient plants. To confirm whether activation of JA-related gene expression led to elevated endogenous hormone contents, we measured MeJA and JA-IIe concentrations in Pi-starved plants over three days. The results showed higher MeJA content in inoculated leaves of Pi-starved plants than in those of the mock control, whereas no significant change of JA-Ile content, the bioactive form in JA signaling, was detected between Pi-starved plants and to Pi-sufficient plants (Figure 2c and S1a). Interestingly, the expression of *OsMYC2,* the key regulator in JA signaling, was also altered upon Pi starvation (Figure 2a and b), gradually increasing in a time-course manner during Pi

starvation (Figure 2d). Together, these results suggested that the activation of *OsMYC2* expression by Pi starvation, not the increase in JA-Ile content, was due to the interaction of Pi starvation and JA signaling.

3.3 *OsMYC2* is a direct target of OsPHR2

OsPHR2 and OsMYC2 are well-known as the central transcription regulators in Pi starvation and JA signaling, respectively (Zhou *et al.* , 2008; Kazan & Manners, 2013). Interestingly, bioinformatics analysis identified three P1BS *cis* -elements in the 2-kb promoter region of *OsMYC2* (Figure 3a). Thus, we first performed a yeast one-hybrid (Y1H) assay to examine their interaction. The 2000 bp fragment containing the P1BS elements was used as bait and cloned into a pAbAi reporter vector, while a pGADT7-Rec2-OsPHR2 vector was used as prey. Yeast cells co-transformed with bait and prey were grown on selective media lacking Leu with or without AbA. The assay results demonstrated that OsPHR2 indeed bound to the promoter region of the *OsMYC2* gene (Figure 3b).

We then performed EMSA using purified His-OsPHR2 fusion protein and biotin-labeled probes (P3 and P4 in Figure 3a). OsPHR2 recombinant protein specifically bound the biotin-labeled probes, and this binding could be suppressed by unlabeled probes (Figure 3c). To test whether OsPHR2 activated the *OsMYC2* promoter, we performed transient expression assays with dual effectors. Transient transfection assay revealed OsPHR2-Flag to increase the expression of *ProOsMYC2-LUC* in *N. benthamiana* leaves as compared with GFP-Flag control (Figure 3d). Finally, to determine whether OsPHR2 directly binds the *OsMYC2* promoter *in vivo* , we performed ChIP-qPCR with anti-Flag antibody on transgenic plants expressing *OsPHR2-Ov1* fused with Flag (Zhou *et al.* , 2008). In this assay, OsPHR2 displayed strong binding to the region containing P1BS *cis* -elements, but not to other regions in the *OsMYC2* promoter (Figure 3e). Together, these results suggest OsPHR2 is directly targeted to the *cis* -element in the *OsMYC2* promoter *in vitro* and *in vivo,* and overexpression of OsPHR2 results in up-regulation of *OsMYC2* .

3.4 OsPHR2 confers JA signaling activation and *Xoo* resistance through regulating expression of *OsMYC2*

The data above clearly demonstrated that OsPHR2 activates the transcription of *OsMYC2* ; accordingly, we further dissected the effects of OsPHR2 on the expression of *OsMYC2* -mediated JA-responsive genes and JA production *in planta* . We first examined *OsMYC2* expression in the presence or absence of Pi in *OsPHR2-Ov1* , *phr2* and the corresponding wild-type NIP plants using RT-qPCR. Under the Pi-sufficient condition, we found that *OsMYC2* was up-regulated in *OsPHR2-Ov1* leaves but down-regulated in *phr2* plants compared with NIP plants (Figure 4a). Consistently, similar expression patterns of JA-responsive genes were also observed in the leaves of the tested mutants (Figure 4b-h). In addition, the Pi starvation-triggered induction of JA-responsive genes, including *OsMYC2* , over three days of growth on Pi-deficient solution was enhanced in *OsPHR2-Ov1* plant leaves but suppressed in *phr2* mutants relative to the Pi-sufficient condition (Figure 4a-h). These results indicate that OsPHR2 positively modulates the expression of JA-responsive genes.

Furthermore, MeJA content was higher in *OsPHR2-Ov1* plants but lower in *phr2* mutants compared with NIP control, whereas only small changes was observed for JA-IIe (Figure 5a and S1b). We also tested the resistance of each type to *Xoo* infection. At 9 to 15 days after inoculation with virulent *Xoo* , bacterial proliferation was lower in *OsPHR2-Ov1* plants but higher in *phr2* plants than in NIP control (Figure 5b). Likewise, blight lesion length was significantly shorter in *OsPHR2-Ov1* plants but longer in *phr2* mutants than in NIP plants (Figure 5c and d), indicating a positive role of OsPHR2 during *Xoo* infection in rice. Taking together, our results indicated that the Pi starvation activated *OsMYC2* expression, which resulted in the induction of MYC2-regulated JA-responsive genes and the increased MeJA production, and thus promoted resistance to *Xoo* infection in rice.

3.5 Involvement of on *OsMYC2* in Pi starvation-enhanced JA signaling and *Xoo* resistance

To further discern whether *OsMYC2* is involved in Pi starvation-induced *Xoo* resistance, we generated *OsMYC2* mutants using the CRISPR/Cas9 system. Two homozygous lines (*myc2-3* and *myc2-6*) were ob-

tained and confirmed by sequencing (Figure S2a). To investigate JA sensitivity, the *OsMYC2* CRISPR/Cas9 mutants and NIP plants were grown with added MeJA. As expected, the transgenic lines displayed less sensitivity to MeJA than the WT plants (Figure S2b and c), indicating *OsMYC2* is required for JA signaling in rice.

To investigate the role of *OsMYC2* in Pi starvation signaling, we first measured the expression of *OsMYC2* -mediated JA response genes in *myc2* plants upon Pi starvation. As shown in Figure 6(a), JA-responsive genes were significantly more highly expressed in Pi-starved NIP plants than in the Pi-sufficient control. In *myc2-3* and *myc2-6* plants, however, Pi deficit suppressed these Pi starvation-inducible JA-responsive genes, except for *OsMYC2* itself (Figure 6a). Phytohormone measurement revealed that the production of MeJA in response to Pi starvation was abolished in *myc2* mutants (Figure 6b). JA-IIe content was lower in *myc2* mutants compared with NIP control, and the level of JA-IIe was not changed upon Pi starvation in both *myc2* and NIP plants (Figure S1c). We also performed *Xoo* resistance tests on these transgenic lines grown under Pi sufficiency or starvation. 9-15 days after inoculated with *Xoo* , we measured bacterial proliferation of plants grown in the normal condition. The results showed that the sensitivity to *Xoo* in *myc2-3* and *myc2-6* plants was higher than that of in NIP controls (Figure 6c). However, Pi starvation-treated *myc2-3* and *myc2-6* plants showed proliferation comparable to that in the control, whereas proliferation in Pi starvation-treated wild-type NIP plants was significantly reduced (Figure 6c). Blight lesion length displayed similar patterns as observed for bacterial population in *myc2-3* and *myc2-6* plants compared with NIP controls under both Pi-sufficient and -deficient conditions (Figure 6d and e), indicating a role for *OsMYC2* in the Pi starvation-enhanced defense response. Thus, our results suggest that *OsMYC2* is necessary for Pi starvation-induced enhancement of the JA signaling and defense response.

4 Discussion

Phosphorus and phytohormones play pivotal roles in regulating diverse developmental and physiological processes of plants (Raghothama & Karthikeyan, 1999; Vidhyasekaran, 2015). Pi starvation signaling crosstalks with hormone pathways to appropriately adapt Pi homeostasis in response to changing environmental conditions (Yuan & Dong, 2008; Baek *et al.* , 2017). Transcriptomic studies have revealed changes in the expression of hormone-responsive genes under Pi starvation in *Arabidopsis* (Hammond *et al.* , 2003; Misson *et al.* , 2005; Bustos *et al.* , 2010; Woo *et al.* , 2012) and in rice (Wasaki *et al.* , 2006; Li *et al.* , 2010; Secco *et al.* , 2013). Pi starvation enhances plant sensitivity to auxin through overexpression of the auxin receptor TIR1 and the polar transport inhibitor BFA, with subsequent modification of the root system (López-Bucio *et al.* , 2000; Nacry *et al.* , 2005; Pérez-Torres *et al.* , 2008). Pi starvation also suppresses cytokinin levels via reducing expression of the cytokinin receptor *CRE1* , thus decreasing plant sensitivity to cytokinin and root length (Martin *et al.* , 2000; Franco-Zorrilla *et al.* , 2002; López-Bucio *et al.* , 2002). Pi starvation additionally suppresses gene expression of enzymes involved in GA metabolism and increases accumulation of the negative regulator protein DELLA to decrease the level of bioactive GA (Jiang *et al.* , 2007). Pi starvation also regulates the transport, synthesis, and catabolism of abscisic acid during changes of the root system architecture (Jaschke *et al.* , 1997; Ciereszko & Kleczkowski, 2006). Notably, interaction between Pi starvation signaling and hormones is also involved in plant defense systems; for example, Pi starvation has recently been demonstrated to induce the JA pathway and enhance resistance to insect herbivory in dicot *Arabidopsis* (Khan *et al.* , 2016). Here, we demonstrated that adaptation to Pi starvation in monocot rice resulted in enhanced bacterial resistance through activation of the JA response (Figures. 1 and 2).

Specifically, we demonstrated that transcriptional regulation of *OsMYC2* by OsPHR2 was integral to Pi starvation-induced promotion of the JA signaling. In Pi starvation signaling, PHRs are known to regulate PSI genes via binding to P1BS elements (Rubio *et al.* , 2001; Zhou *et al.* , 2008; Bustos *et al.* , 2010). *AtPHR1* partially controls Pi deficiency-triggered induction of JA signaling in *Arabidopsis* (Khan *et al.* , 2016), but the molecular mechanism remains to be elucidated. Here, we identified three P1BS *cis* -elements in the 2-kb promoter region of *OsMYC2* and further confirmed that OsPHR2, the homolog of AtPHR1 and thus the central regulator in rice (Wu *et al.* , 2013), was directly targeted to the promoter of *OsMYC2* both *in vivo* and *in vitro* (Figure 3). Expression of *OsMYC2* was enhanced in *OsPHR2* overexpression mutants but sup-

pressed in *OsPHR2*T-DNA insertion mutants grown under normal condition (Figure 4), further confirming expression of *OsMYC2* as controlled by OsPHR2. Moreover, both the expression patterns of JA-synthesis genes and the basal MeJA level behaved in a similar manner as *OsMYC2* in*OsPHR2-Ov1* and *phr2* mutants (Figures 4 and 5); this is consistent with the previous finding in *Arabidopsis* that MYC2/MYC3/MYC4 directly control the water spray-induced accumulation of JA (Van Moerkercke *et al.* , 2019). In this work, we additionally demonstrated that in *myc2* mutants, neither the expression of JA-responsive genes nor MeJA production were altered in either the presence or absence of Pi (Figure 6), suggesting that activation of the JA signaling by Pi starvation depends on *OsMYC2* . JA-IIe is the bioactive form in JA signaling, we also noticed the JA-IIe was not changed upon Pi starvation treatment or in *OsPHR2* mutants (Figure S1). However, evidences have shown that JA signaling is clearly activated by repeated touching, wounding and oral secretion or short-term exposure to gaseous NO_2 while JA-Ile levels remained unchanged in *Arabidopsis* (Chehab *et al* . 2012; Lange and Lange 2015; Bozorov et al., 2017; Mayer *et al* . 2018), supporting the notion that JA signaling can turn on without measurable increase in JA-Ile (Thierry *et al* ., 2019). Thus, we speculate that the transcriptional regulation of *OsMYC2* by OsPHR2 reveals direct crosstalk between JA and Pi starvation signaling at the molecular level.

Prior studies have revealed that activation of JA signaling plays a positive role in *Xoo* resistance in rice (Koeduka *et al.* , 2005; Tao *et al.* , 2009; Deng *et al.* , 2012; Yamada *et al.* , 2012; Uji *et al.* , 2016; Ke *et al.* , 2020; Onohata & Gomi, 2020). For example, overexpressing *OsWRKY45* and*OsC3H12* enhanced *Xoo* resistance, accumulation of JA, and expression of JA signaling genes (Tao *et al.* , 2009; Deng *et al.* , 2012). Exogenous application of JA also enhanced resistance to bacterial blight, and this JA-induced *Xoo* resistance could be inhibited by overexpressing *OsJAZ8[?]C* , which lacks the Jas domain (Yamada *et al* ., 2012) In the present work, we observed Pi starvation to enhance resistance to *Xoo* , accompanied by elevated expression of JA-responsive genes and accumulation of MeJA (Figures 1 and 2). In addition, we found that *OsPHR2-Ov1* mutants (which have an activated JA signaling with unchanged JA-IIe level) were more resistant while *phr2* mutants (which have a suppressed JA signaling) were more susceptible compared with the wild-type NIP plants (Figures 4, 5 and S1). Nevertheless, repetitive mechanical stimulation and NO_2 fumigation enhances *Arabidopsis*resistance to *Botrytis cinerea* with activated JA signaling in JA-IIe steady-stage levels (Chehab *et al* . 2012; Mayer *et al* . 2018). Evidences also show that JA-IIe is not required to activate JA mediated systemic defenses to herbivory in *Nicotiana attenuate*and *Solanum nigrum* (Doorn et al., 2011; Bozorov et al., 2017). Therefore, we speculated that the JA signaling is involved in OsPHR2-mediated anti-*Xoo* defense. In*Arabidopsis* , *AtMYC2* and *AtERF3* antagonistically repress JA-induced pathogen defense genes, and thus *myc2-2*mutants show increased sensitivity to the necrotrophic pathogen*Botrytis cinerea* (Lorenzo *et al.* , 2004; Zhai *et al.* , 2013). In tomato, however, *MYC2* -silenced plants display enhanced resistance to *Botrytis cinerea* , as MYC2 and MTF ETHYLENE RESPONSE FACTOR.C3 synergistically and preferentially modulate pathogen-responsive genes (Du *et al.* , 2017). In rice, transgenic plants overexpressing *OsMYC2* display a JA-hypersensitive phenotype and are more resistant to *Xoo* (Uji *et al.* , 2016); meanwhile, in *OsMYC2* RNAi plants, the JA-inducible expression of many defense-related genes and JA-dependent activation of the biosynthetic pathways for specialized metabolites are both compromised (Ogawa *et al.* , 2017). Here, our data revealed*OsMYC2* CRISPR/Cas9 mutants to exhibit a JA-insensitive phenotype and greater susceptibility to *Xoo* infection (Figure 6). In addition, the *Xoo*susceptibility of *myc2* lines was not enhanced by Pi starvation (Figure 6), suggesting involvement of *OsMYC2* in Pi starvation-mediated *Xoo* resistance. Considering OsPHR2 physically binds to the *OsMYC2* promoter to regulate *OsMYC2*expression, resulting in consequent activation of the JA signaling (Figures. 3-5), we speculated that activation of the JA response resulting from the transcriptional regulation of *OsMYC2* by OsPHR2 contributes, at least partially, to the bacterial defense induced by Pi starvation.

During its growth and development, rice is confronted by simultaneous nutrition deficiency and pathogen attack, such as Pi starvation (or low Pi) and bacterial blight. Here, we found a positive effect of Pi starvation on resistance to *Xoo* . Together with the well-known SPXs-PHR1 working model of the Pi starvation signaling pathway, we propose a model illustrating that Pi starvation- and JA- signaling function synergistically and positively to control rice resistance to*Xoo* infection (Figure 7). When grown under the Pi-sufficient condition,

OsSPXs interact with OsPHR2 with high binding affinity, prevent its binding to the P1BS motifs in the promoter of *OsMYC2* , thus *OsMYC2* is expressed at a solely basal level. Under the Pi-starvation condition, weakened interaction of OsSPXs-PHR2 allows PHR2 to up-regulate *OsMYC2* , thereby enhancing expression of*OsMYC2* and consequently activating the JA response and JA-mediated antibacterial resistance. Our findings reveal a novel mechanism for crosstalk between Pi-starvation signaling and the JA pathway and its positive role in rice antibacterial immunity, and provide new insight into how plants adjust the balance between growth and defense by integrating nutrient supply and phytohormone signaling.

Support information

Figure S1. Quantification of JA-IIe levels.

Figure S2. Identification of *myc2* mutants.

Table S1. Primers used in this study.

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Author Contribution

Y.H. and G.H. conceived the project and designed the experiments; Y.K. G.W and Y.H. carried out the experiments with assistance from X.C., L.L., and X.Z.; all authors analyzed and discussed the results; and Y.H. and G.H. wrote the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Agrawal GK, Jwa N, Rakwal R. 2000a. A novel rice *(Oryza sativa L .)* acidic *OsPR1a* gene highly responsive to cut, phytohormones, and protein phosphatase inhibitors. *Biochemical and Biophysical Research Communications* **274** (1): 157-165.

Agrawal GK, Rakwal R, Jwa N. 2000b. Rice (*Oryza sativa L.*) *OsPR1b* gene is phytohormonally regulated in close interaction with light sgnals. *Biochemical and Biophysical Research Communications* **278** (2): 290-298.

Aurelie G, Robin L, Farmer EE. 2010. *Arabidopsis*jasmonate signaling pathway. *Science Signaling* **3** (109): cm4.

Baek D, Chun HJ, Yun DJ, Min CK. 2017. Cross-talk between phosphate starvation and other environmental stress signaling pathways in plants. *Moleculer Cells* **40** (10): 697-705.

Bozorov TA, Dinh ST, Baldwin IT. 2017. JA but not JA-Ile is the cell-nonautonomous signal activating JA mediated systemic defenses to herbivory in Nicotiana attenuata. *Journal of Integrative Plant Biology* **59** (8): 552-571.

Bustos R, Castrillo G, Linhares F, Puga MI, Rubio V, Perez-Perez J, Solano R, Leyva A, Paz-Ares J. 2010. A central regulatory system largely controls transcriptional activation and repression responses to phosphate ptarvation in *Arabidopsis* . *Plos Genetics***6** (9): e1001102.

Chehab EW, Yao C, Henderson Z, Kim S, Braam J. 2012.*Arabidopsis* touch-induced morphogenesis is jasmonate mediated and protects against pests. *Current Biology* **22** (8): 701–706.

Chen X, Sun C, Laborda P, He Y, Zhao Y, Li C, Liu F. 2018. Melatonin treatments reduce the pathogenicity and inhibit the growth of Xanthomonas oryzae pv. oryzicola. *Plant Pathology* **68** : 288-296.

Chini A, Fonseca S, Chico JM, Fernandez-Calvo P, Solano R. 2009. The ZIM domain mediates homo- and heteromeric interactions between Arabidopsis JAZ proteins. *Plant Journal* **59** (1): 77-87.

Chini A, Fonseca S, Fernandez G, Adie B, Chico J, Lorenzo O, GarciaCasado G, LopezVidriero I, Lozano F, Ponce M. 2007. The JAZ family of repressors is the missing link in jasmonate signalling.*Nature* **448** (7154): 666-671.

Chini A, Gimenez-Ibanez S, Goossens A, Solano R. 2016. Redundancy and specificity in jasmonate signalling. *Current Opinion in Plant Biology* **33** : 147-156.

Ciereszko I, Kleczkowski LA. 2006. Phosphate deficiency dependent upregulation of UDP-glucose pyrophosphorylase genes is insensitive to ABA and ethylene status in *Arabidopsis* leaves.*Acta Physiologiae Plantarum* **28** (5): 387-393.

Cong W, Suriyagoda L, Lambers H. 2020. Tightening the phosphorus cycle through phosphorus-efficient crop genotypes.*Trends in Plant Science* .

Crombez H, Motte H, Beeckman T. 2019. Tackling plant phosphate starvation by the roots. *Developmental Cell* **48** (5): 599-615.

Deng H, Liu H, Li X, Xiao J, Wang S. 2012. A CCCH-type zinc finger nucleic acid-binding protein quantitatively confers resistance against rice bacterial blight disease. *Plant Physiology***158** (2): 876-889.

Dombrecht B, Xue GP, Sprague SJ, Kirkegaard JA, Ross JJ, Reid JB, Fitt GP, Sewelam N, Schenk PM, Manners JM. 2007. MYC2 differentially modulates diverse jasmonate-dependent functions in*Arabidopsis* . *Plant Cell* **19** (7): 2225-2245.

Doorn AV, Bonaventure G, Rogachev I, Aharoni A, Baldwin IT. 2011. JA-Ile signaling in Solanum nigrum is not required for defense responses in nature. 2011. *Plant Cell & Envroment***34** (12): 2159-2171.

Du M, Zhao J, Tzeng DTW, Liu Y, Li C. 2017. MYC2 orchestrates a hierarchical transcriptional cascade that regulates Jasmonate-mediated plant immunity in tomato. *Plant Cell* **29** (8): 1883–1906.

Franco-Zorrilla JM, Gonzalez E, Bustos R, Linhares F, Leyva A, Paz-Ares J. 2004. The transcriptional control of plant responses to phosphate limitation. *J Exp Bot* **55** (396): 285-293.

Franco-Zorrilla JM, Martin AC, Solano R, Rubio V, Paz-Ares J. 2002. Mutations at CRE1 impair cytokinin-induced repression of phosphate starvation responses in *Arabidopsis* . *Plant Journal* **32** (3): 353-360.

Fu J, Chu J, Sun X, Wang J, Yan C. 2012. Simple, rapid, and simultaneous assay of multiple carboxyl containing phytohormones in wounded tomatoes by UPLC-MS/MS using single SPE purification and isotope dilution. *Analytical Sciences* **28** (11): 1081-1087.

Guo M, Ruan W, Li C, Huang F, Zeng M, Liu Y, Yu Y, Ding X, Wu Y, Wu Z. 2015. Integrative comparison of the role of the PHOSPHATE RESPONSE1 subfamily in phosphate signaling and homeostasis in rice.*Plant Physiology* **168** (4): 1762-1776.

Hammond JP, Bennett MJ, Bowen HC, Broadley MR, Eastwood DC, May ST, Rahn C, Swarup R, Woolaway KE, White PJ. 2003. Changes in gene expression in *Arabidopsis* shoots during phosphate starvation and the potential for developing smart plants. *Plant Physiology***132** (2): 578-596.

He Y, Hong G, Zhang H, Tan X, Li L, Kong Y, Sang T, Xie K, Wei J, Li J, et al. 2020. The OsGSK2 Kinase Integrates Brassinosteroid and Jasmonic Acid Signaling by Interacting with OsJAZ4. *The Plant Cell* **32** (9): 2806-2822.

He Y, Zhang H, Sun Z, Li J, Hong G, Zhu Q, Zhou X, Macfarlane S, Yan F, Chen J. 2017. Jasmonic acid-mediated defense suppresses brassinosteroid-mediated susceptibility to Rice black streaked dwarf virus infection in rice. *New Phytologist* **214** (1): 388-399.

He Y, Zhang X, Li L, Sun Z, Li J, Chen X, Hong G. 2021. SPX4 interacts with both PHR1 and PAP1 to regulate critical steps in phosphorus-status-dependent anthocyanin biosynthesis. *New Phytologist* **230** (1): 2015-217.

Hong G-J, Xue X-Y, Mao Y-B, Wang L-J, Chen X-Y. 2012.*Arabidopsis* MYC2 interacts with DELLA proteins in regulating sesquiterpene synthase gene expression. *Plant Cell***24** (6): 2635-2648.

Howe GA, Major IT, Koo AJ. 2018. Modularity in jasmonate signaling for multistress resilience. *Annual Review of Plant Biology* **69** (1): annurev-arplant-042817-040047.

Jaschke WD, Peuke AD, Pate JS, Hartung W. 1997. Transport, synthesis and catabolism of abscisic acid (ABA) in intact plants of castor bean (*Ricinus communis* L.) under phosphate deficiency and moderate salinity. *Journal of Experimental Botany* **48** (9): 1737-1747.

Jiang C, Gao X, Liao L, Harberd NP, Fu X. 2007. Phosphate starvation root architecture and anthocyanin cccumulation responses are modulated by the gibberellin-DELLA signaling pathway in*Arabidopsis* . *Plant Physiology* **145** (4): 1460-1470.

Kauffman HE, Reddy APK, Hsieh SPY, Merca SD. 1973. An improved technique for evaluating resistance of rice varieties to Xanthomonas oryzae. *Plant disease reporter* **57** (6): 537-541.

Kazan K, Manners JM. 2011. The interplay between light and jasmonate signalling during defence and development. *Journal of Experimental Botany* **62** (12): 4087-4100.

Kazan K, Manners JM. 2013. MYC2: The master in action.*Molecular Plant* **6** (3): 686-703.

Ke Y, Hui S, Yuan M. 2017. *Xanthomonas oryzae* pv.*oryzae* inoculation and growth rate on rice by leaf clipping method. *Bio-Protocol* **7** (19).

Ke Y, Yuan M, Liu H, Hui S, Qin X, Chen J, Zhang Q, Li X, Xiao J, Zhang Q, Li X, Xiao J, Zhang Q, Wang SP. 2020. The versatile functions of OsALDH2B1 provide a genic basis for growth-defense trade-offs in rice. *Proceedings of the National Academy of Sciences of the United States of America* **117** (7): 3867-3873.

Khan GA, Vogiatzaki E, Glauser G, Poirier Y. 2016. Phosphate deficiency induces the jasmonate pathway and enhances resistance to insect herbivory. *Plant Physiology* **171** (1): 632-644.

Koeduka T, Matsui K, Hasegawa M, Akakabe Y, Kajiwara T. 2005. Rice fatty acid α α math-Container Loading Mathjax -dioxygenase is induced by pathogen attack and heavy metal stress: activation through jasmonate signaling. *Journal of Plant Physiology* **162** (8): 912-920.

Kramell R, Miersch O, Wasternack C, Solano R, Fonseca S, Chini A, Hamberg M, Adie B, Porzel A. 2009. (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. *Nature Chemical Biology***5** (5): 344-350.

Lange MJ, Lange T. 2015. Touch-induced changes in *Arabidopsis* morphology dependent on gibberellin breakdown. *Nature Plants* **1** (3): 14025.

Li L, Liu C, Lian X. 2010. Gene expression profiles in rice roots under low phosphorus stress. *Plant molecular biology***72** (4-5): 423-432.

Liu F, Wang Z, Ren H, Shen C, Li Y, Ling H, Wu C, Lian X, Wu P. 2010. OsSPX1 suppresses the function of OsPHR2 in the regulation of expression of *OsPT2* and phosphate homeostasis in shoots of rice.*Plant Journal* **62** (3): 508-517.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-[?][?]C}$ tMethod. *Methods* **25** (4): 402-408.

López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L. 2003. The role of nutrient availability in regulating root architecture.*Current Opinion in Plant Biology* **6** (3): 280-287.

López-Bucio J, Hernandezabreu E, Sanchezcalderon L, Nietojacobo MF, Simpson J, Herrera-estrella L. 2002. Phosphate availability alters architecture and causes changes in hormone sensitivity in the*Arabidopsis* root system. *Plant Physiology* **129** (1): 244-256.

López-Bucio J, Vega OMDL, Guevara-García A, Herrera-Estrella L. 2000. Enhanced phosphorus uptake in tobacco transgenic plants that overproduce citrate. . *Nat Biotechnology* **18** (4): 450-453.

Lorenzo O, Chico J, Sa´nchez-Serrano J, Solano R. 2004.JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in*Arabidopsis* . *Plant Cell* **16** (7): 1938-1950.

Lv Q, Zhong Y, Wang Y, Wang Z, Zhang L, Shi J, Wu Z, Liu Y, Mao C, Yi K, et al. 2014. SPX4 Negatively Regulates Phosphate Signaling and Homeostasis through Its Interaction with PHR2 in Rice. *Plant Cell***26** (4): 1586-1597.

Martin AC, Pozo JCD, Iglesias J, Rubio V, Solano R, La Pena AD, Leyva A, Pazares J. 2000. Influence of cytokinins on the expression of phosphate starvation responsive genes in *Arabidopsis* . *Plant Journal* **24** (5): 559-567.

Mayer D, Mithofer A, Glawischnig E, Georgii E, Ghirardo A, Kanawati B, Schnitzler JP, Durner J, Gaupels F. 2018. Short-term exposure to nitrogen dioxide provides basal pathogen resistance. *Plant Physiology* **178** (1): 468–487.

Miao J, Guo D, Zhang J, Huang Q, Qin G, Zhang X, Wan J, Gu H, Qu L. 2013. Targeted mutagenesis in rice using CRISPR-Cas system.*Cell Research* **23** (10): 1233-1236.

Misson J, Raghothama KG, Jain A, Jouhet J, Block MA, Bligny R, Ortet P, Creff A, Somerville S, Rolland N. 2005. A genome-wide transcriptional analysis using *Arabidopsis thaliana* Affymetrix gene chips determined plant responses to phosphate deprivation.*Proceedings of the National Academy of Sciences of the United States of America* **102** (33): 11934-11939.

Nacry P, Canivenc G, Muller B, Azmi A, Onckelen H, Rossignol M, Doumas P. 2005. A role for Auxin redistribution in the responses of the root system architecture to phosphate starvation in *Arabidopsis* .*Plant Physiology* **138** (4): 2061-2074.

Neumann G, RÖmheld V. 2012. *Rhizosphere chemistry in relation to plant nutrition. In Marschner's Mineral Nutrition of Higher Plants, 3rd edn, H. Marschner, ed. London: Academic Press)* .

Niu YF, Chai RS, Jin GL, Wang H, Tang CX, Zhang YS. 2013. Responses of root architecture development to low phosphorus availability: a review. *Annual Botany* **112** (2): 391-408.

Ogawa S, Kawahara-Miki R, Miyamoto K, Yamane H, Nojiri H, Tsujii Y, Okada K. 2017. OsMYC2 mediates numerous defence-related transcriptional changes via jasmonic acid signalling in rice.*Biochemical & Biophysical Research Communications***486** (3): 796-803.

Onohata T, Gomi K. 2020. Overexpression of jasmonate-responsive OsbHLH034 in rice results in the induction of bacterial blight resistance via an increase in lignin biosynthesis. *Plant Cell Reports* .

Pauwels L, Barbero GF, Geerinck J, Tilleman S, Grunewald W, Pérez AC, Chico JM, Bossche RV, Sewell J, Gil E. 2010. NINJA connects the co-repressor TOPLESS to jasmonate signalling. *Nature***464** (8): 788-791.

Pérez-Torres CA, Lopez-Bucio J, Cruz-Ramirez A, Ibarra-Laclette E, Dharmasiri S, Estelle M, Herrera-Estrella L. 2008. Phosphate availability alters lateral root development in *Arabidopsis* by modulating auxin sensitivity via a mechanism involving the TIR1 Auxin receptor. *Plant Cell* **20** (12): 3258-3272.

Puga MI, Isabel M, Rajulu C, Zhiye W, Franco-Zorrilla JM, Laura DL, Irigoyen ML, Simona M, Regla B, José R. 2014. SPX1 is a phosphate-dependent inhibitor of Phosphate Starvation Response 1 in Arabidopsis. *Proc Natl Acad Sci U S A* **111** (41): 14947-14952.

Raghothama KG, Karthikeyan AS. 1999. Phosphate Acquisition.*Annual Review of Plant Physiology & Plant Molecular Biology***274** (1-2): 37-49.

Rakwal R, Komatsu S. 2000. Role of jasmonate in the rice (*Oryza sativa L.)* self-defense mechanism using proteome analysis.*Electrophoresis* **21** (12): 2492-2500.

Rohit D, Preshobha KP, Michael R. 2016. Functional Analysis of Jasmonates in Rice through Mutant Approaches. *Plants***5** (1): 15.

Ruan J, Zhou Y, Zhou M, Yan J, Khurshid M, Weng W, Cheng J, Zhang K. 2019. Jasmonic acid signaling pathway in plants.*International Journal of Molecular Sciences* **20** (10): 2479.

Ruan W, Meina G, Wu P, Yi KK. 2016. Phosphate starvation induced OsPHR4 mediates Pi-signaling and homeostasis in rice. **93** (3): 1-14.

Ruan W, Guo M, Wang X, Guo Z, Xu Z, Xu L, Zhao H, Sun H, Yan C, Yi K. 2019. Two RING-finger ubiquitin E3 ligases regulate the degradation of SPX4, an internal phosphate sensor, for phosphate homeostasis and signaling in rice. *Molecular plant***12** (8): 1060-1074.

Ruan W, Guo M, Wu P, Yi K. 2017. Phosphate starvation induced OsPHR4 mediates Pi-signaling and homeostasis in rice. *Plant molecular biology* **93** (3): 1-14.

Rubio V, Linhares F, Solano R, Martin AC, Iglesias J, Leyva A, Paz-Ares J. 2001. A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes Dev* **15** (16): 2122-2133.

Secco D, Jabnoune M, Walker H, Shou H, Whelan J. 2013. Spatio-temporal transcript profiling of rice roots and shoots in response to phosphate starvation and recovery. *Plant Cell***25** (11): 4285-4304.

Tao Z, Liu H, Qiu D, Zhou Y, Wang S. 2009. A pair of allelic WRKY genes play opposite roles in rice-bacteria interactions.*Plant Physiology* **151** (2): 936-948.

Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He SY, Howe GA, Browse J. 2007. JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. *Nature***448** (7154): 661-665.

Thierry H, Ekaterina S, Valentin M, Laure P. 2019. Metabolic control within the jasmonate biochemical pathway. *Plant & Cell Physiology* , **60** (12): 2621-2628.

Uji Y, Taniguchi S, Tamaoki D, Shishido H, Akimitsu K, Gomi K. 2016. Overexpression of *OsMYC2* results in the upregulation of early JA-responsive genes and bacterial blight resistance in rice.*Plant & Cell Physiology* **57** (9): pcw101.

Van Moerkercke A, Duncan O, Zander M, Šimura J, Broda M, Vanden Bossche R, Lewsey M, Lama S, Singh K, Ljung K, et al. 2019. A MYC2/MYC3/MYC4-dependent transcription factor network regulates water spray-responsive gene expression and jasmonate levels. *Proceedings of the National Academy of Sciences of the United States of America***116** (46): 23345-23356.

Vidhyasekaran P. 2015. *Plant hormone signaling systems in plant innate immunity.* : Springer Netherlands.

Wang Z, Ruan W, Shi J, Zhang L, Xiang D, Yang C, Li C, Wu Z, Liu Y, Yu Y, et al. 2014. Rice SPX1 and SPX2 inhibit phosphate starvation responses through interacting with PHR2 in a phosphate-dependent manner.*Proceedings of the National Academy of Sciences of the United States of America* **111** (41): 14953-14958.

Wasaki J, Shinano T, Onishi K, Yonetani R, Yazaki J, Fujii F, Shimbo K, Ishikawa M, Shimatani Z, Nagata Y, et al. 2006. Transcriptomic analysis indicates putative metabolic changes caused by manipulation of phosphorus availability in rice leaves. *Journal of Experimental Botany* **57** (9): 2049-2059.

Wasternack C, Hause B. 2013. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. *Annals of Botany* **111** (6): 1021-1058.

Wild R, Gerasimaite R, Jung JY, Truffault V, Pavlovic I, Schmidt A, Saiardi A, Jessen HJ, Poirier Y, Hothorn M. 2016. Control of eukaryotic phosphate homeostasis by inositol polyphosphate sensor domains. *Science* **352** (6288): 986.

Woo J, MacPherson C, Liu J, Wang H, Kiba T, Hannah MA, Wang X-J, Bajic VB, Chua N-H. 2012. The response and recovery of the*Arabidopsis thaliana* transcriptome to phosphate starvation.*Bmc Plant Biology* **12** (1): 62.

Wu P, Shou H, Xu G, Lian X. 2013. Improvement of phosphorus efficiency in rice on the basis of understanding phosphate signaling and homeostasis. *Current Opinion in Plant Biology* **16** (2): 205-212.

Yamada S, Kano A, Tamaoki D, Miyamoto A, Shishido H, Miyoshi S, Taniguchi S, Akimitsu K, Gomi K. 2012. Involvement of OsJAZ8 in Jasmonate-Induced Resistance to Bacterial Blight in Rice. *Plant & Cell Physiology* **53** (12): 2060.

Yang B, Bogdanove A. 2013. Inoculation and virulence assay for bacterial blight and bacterial leaf streak of rice. *Methods in molecular biology (Clifton, N.J.)* **956** : 249-255.

Yang D, Yao J, Mei C, Tong X, Zeng L, Li Q, Xiao L, Sun T, Li J, Deng,XingWang. 2012. Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade.*Proceedings of the National Academy of Sciences of the United States of America* **109** (19): E1192.

Yoshida S, Forno D, Cock J, Gomez K. 1976. Laboratory manual for physiological studies of rice, Ed 3rd. *The International Rice Research Institute,Manila, Philippines* .

Yuan H, Dong D. 2008. Signaling components involved in plant responses to phosphate starvation. *Journal of Integrative Plant Biology* **50** (7): 849-859.

Zhai Q, Yan L, Tan D, Chen R, Sun J, Gao L, Dong M-Q, Wang Y, Li C, Yu H. 2013. phosphorylation-coupled proteolysis of the transcription factor MYC2 is important for jasmonate-signaled plant immunity.*Plos Genetics* **9** (4): e1003422.

Zhong Y, Wang Y, Guo J, Zhu X, Shi J, He Q, Liu Y, Wu Y, Zhang L, Lv Q. 2018. Rice SPX6 negatively regulates the phosphate starvation response through suppression of the transcription factor PHR2. *New Phytologist* **219** (1): 135-148.

Zhou J, Jiao F, Wu Z, Li Y, Wang X, He X, Zhong W, Wu P. 2008. OsPHR2 is involved in phosphate-starvation signaling and excessive phosphate accumulation in shoots of plants. *Plant Physiology***146** (4): 1673-1686.

Figure legends

Figure 1. Phosphate (Pi) starvation confers rice resistance to*Xanthomonas oryzae* pv. *oryzae* (*Xoo*) infection.

(a) Growth of *Xoo* strain PXO91 in wild-type Nipponbare (NIP) leaves upon Pi starvation treatment. Plants were grown with or without Pi solution for three days and then inoculated with *Xoo* . Leaves clipped by dipping scissor tips in sterilized water were used as control. The bacterial population was measured from three leaves at each time point in terms of colony-forming units (cfu). Leaf fragments (6 cm) were sterilized using 75% ethanol, ground separately, suspended in sterilized water, and plated on peptone sucrose agar in a 10-fold dilution series. Different letters indicate significant difference at $P < 0.05$ by Fisher's least significant difference tests. (b) Bacterial blight symptoms in wild-type NIP leaves at 14 days after inoculation with *Xoo* . (c) Lesion length on the fifth leaf blades at 14 days after inoculation with *Xoo* . Values are means ± SE (n = 15). * $P< 0.05$, Student's t -test.

Figure 2. Phosphate (Pi) starvation induces JA-responsive gene expression and MeJA production.

(a and b) RT-qPCR analysis of JA-responsive genes in wild-type Nipponbare (NIP) plants upon Pi starvation treatment for 12 hours (a) and three days (b), respectively. Ten-day-old NIP plants were grown in +P/-P solution and the treated leaves were collected at 12 hours and three days for RNA extraction. *OsIPS1* (*Induced by phosphate starvation 1*) is a marker gene for Pi starvation. Values are means ± SE of three biological replicates. * indicates significant difference between +P/-P treatment at $P < 0.05$ by Student'st -test. H: hours; D: day. (c) Enhanced levels of endogenous MeJA in seven-day-old NIP plants upon Pi starvation treatment for three days. The limit of quantification for MeJA was 22.3 nM. Values are means ± SE of three biological replicates. * $P < 0.05$, Student'st -test. (d) Expression of *OsMYC2* in NIP leaves upon starvation treatment. Ten-day-old plants were grown in solution with an additional time course of phosphate starvation and phosphate recovery (RP) afterwards. Treated leaves were used for RNA extraction at the indicated time after treatment. Values are means ± SE of three biological replicates. * $P < 0.05$, Student'st -test.

Figure 3. OsPHR2 targets the *OsMYC2* promoter. (a) Diagram of the *OsMYC2* promoter and its OsPHR2 binding sites (GNATATNC). Black lines P1–5 indicate the sequences tested in ChIP assays. P1 contained GAATATAC, P2 contained GTATATAC, and P3 contained GCATATGC; those elements were absent in P4 and P5. (b) Yeast one-hybrid assays showing interaction between OsPHR2 and *OsMYC2* promoter fragments. Empty pGADT7-Rec2 vectors were used as negative control. AbA: Aureobasidin A. (c) Gel shift assay indicating OsPHR2 protein binds to the *OsMYC2* promoter *in vitro* . The arrow indicates band shifting caused by OsPHR2 binding to the P3 and P4 motifs of*OsMYC2* promoter, labeled with biotin (hot probe). The competitive protein-DNA binding assay was performed using 10X and 100X unlabeled probes of the wild-type (cold probe). (d) Transient transfection assay indicated that OsPHR2 activated *OsMYC2* promoter in *N. benthamiana* leaves. (e) ChIP assay revealed OsPHR2 enriched the*OsMYC2* promoter fragment *in vivo* . *OsACTIN2* gene promoter was used as a reference and *OsIPS1* gene promoter was set as positive control. Fold enrichment represents the binding efficiency ratio of anti-Flag antibody/negative IgG antibody. Data are means ± SE (n = 3). * $P < 0.05$, Student's t- test.

Figure 4. OsPHR2 regulates expression of JA-responsive genes. Ten-day-old *OsPHR2-Ov1* , *phr2* , and wild-type Nipponbare (NIP) plants were grown in solution with 0 (-P) or 200 μM (+P) Pi for three days. Treated leaves were used for RNA extraction and transcripts were analyzed by qRT-PCR. *OsIPS1* (*Induced by phosphate starvation 1*) is a marker gene for Pi starvation. Values are means ± SE of three biological replicates. Different letters indicate significant difference at $P < 0.05$ by Fisher's least significant difference tests.

Figure 5. OsPHR2 positively regulates MeJA accumulation and antibacterial defense. (a) Endogenous MeJA levels in ten-day-old*OsPHR2-OV1* , *phr2* , and wild-type Nipponbare (NIP) leaves. The limit of quantification for MeJA was 22.3 nM. Values are means ± SE of three biological replicates. Different letters indicate significant difference at $P < 0.05$ by Fisher's least significant difference (LSD) tests. (b) Growth of *Xanthomonas oryzae* pv.*oryzae* (*Xoo*) strain PXO91 in leaves of *OsPHR2-Ov1* ,*phr2* , and NIP. Plants were grown with or without Pi solution for three days and then inoculated with *Xoo* . Leaves clipped by dipping scissor tips in sterilized water were used as control. The bacterial population was measured from

three leaves at each time point in terms of colony-forming units (cfu). Leaf fragments (6 cm) were sterilized using 75% ethanol, ground separately, suspended in sterilized water, and plated on peptone sucrose agar in a 10-fold dilution series. $P < 0.05$, Fisher's LSD test. (c) Bacterial blight symptoms in NIP, *OsPHR2-Ov1* , and *phr2*plants at 14 days after inoculation with *Xoo* . The fifth leaf of each plant was inoculated with *Xoo* . (d) Lesion length in fifth leaf blades of NIP, *OsPHR2-Ov1* , and *phr2* at 14 days after inoculation with *Xoo* . Values are means ± SE (n [?] 15). $P< 0.05$, Fisher's LSD test.

Figure 6. *OsMYC2* is involved in phosphate (Pi) starvation-induced resistance. (a) Effects of Pi starvation treatment on the expression of JA-responsive genes in 5- to 5.5-stage Nipponbare (NIP), *myc2-3* , and *myc2-6* plants. Treated leaves were used for RNA extraction and transcripts were analyzed by qRT-PCR. Values are means +- SE of three biological replicates. Different letters on the top of column for each gene indicate significant difference at $P< 0.05$ by Fisher's least significant difference (LSD) test. (b) Endogenous MeJA levels in 5- to 5.5-stage wild-type NIP,*myc2-3* , and *myc2-6* leaves treated with Pi starvation solution for three days. The limit of quantification for MeJA was 22.3 nM. $P < 0.05$, Fisher's LSD test. (c) Growth of*Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strain PXO91 in leaves of *myc2-3* , *myc2-6* , and NIP upon Pi starvation. Plants were grown with or without Pi solution for three days and then inoculated with *Xoo* . Leaves clipped by dipping scissor tips in sterilized water were used as control. The bacterial population was measured from three leaves at each time point in terms of colony-forming units (cfu). Leaf fragments (6 cm) were sterilized using 75% ethanol, ground separately, suspended in sterilized water, and plate on peptone sucrose agar in a 10-fold dilution series. $P < 0.05$, Fisher's LSD test. The data in cycle displayed non-significance. (d and e) Bacterial blight symptoms (d) and lesion length (e) in NIP,*myc2-3* , and *myc2-6* plants at 14 days after inoculation with *Xoo* . Values are means +- SE (n [?] 12). $P < 0.05$, Fisher's LSD test.

Figure. 7 Model of the OsPHR2-*OsMYC2*-mediated JA response to bacterial resistance induced by phosphate (Pi) starvation. Under high Pi, OsSPXs interact with OsPHR2 at high affinity and prevent OsPHR2 from binding to the P1BS motifs of *OsMYC2* . Thus, expression of *OsMYC2* is basal. When Pi is low or lacking, the OsPHR2-OsSPXs interaction is low affinity and P1BS motifs compete with OsSPXs for OsPHR2 binding, allowing OsPHR2 to up-regulate *OsMYC2* . Increased *OsMYC2*triggers the JA response and thereby enhances rice antibacterial resistance. Bold lines ending with arrows show activation.