The bacterial effector AvrRxo1 inhibits vitamin B6 biosynthesis to promote infection in rice

Haifeng Liu1,2, Chongchong Lu1, Yang Li1, Tao Wu1, Baogang Zhang1, Baoyou Liu1, Wenjie Feng1, Qian Xu2, Hansong Dong1, Shengyang He3,4,5, Zhaohui Chu6,* and Xinhua Ding1,*

1State Key Laboratory of Crop Biology, Shandong Provincial Key Laboratory for Biology of Vegetable Diseases and Insect Pests, College of Plant Protection, Shandong Agricultural University, Tai an, 271018 Shandong, PR China
2College of Agronomy, Shandong Agricultural University, Tai an, 271018 Shandong, PR China
3Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA
4Department of Biology, Duke University, Durham, NC 27708, USA
5Howard Hughes Medical Institute, Duke University, Durham, NC 27708, USA
6State Key Laboratory of Hybrid Rice, College of Life Sciences, Wuhan University, Wuhan, 430072 Hubei, PR China
*Correspondence: Zhaohui Chu (zchu77@whu.edu.cn), Xinhua Ding (xhding@sdau.edu.cn)

https://doi.org/10.1016/j.xplc.2022.100324

ABSTRACT

Xanthomonas oryzae pv. oryzicola (Xoc), which causes rice bacterial leaf streak, invades leaves mainly through stomata, which are often closed as a plant immune response against pathogen invasion. How Xoc overcomes stomatal immunity is unclear. Here, we show that the effector protein AvrRxo1, an ATP-dependent protease, enhances Xoc virulence and inhibits stomatal immunity by targeting and degrading rice OsPDX1 (pyridoxal phosphate synthase), thereby reducing vitamin B6 (VB6) levels in rice. VB6 is required for the activity of aldehyde oxidase, which catalyzes the last step of abscisic acid (ABA) biosynthesis, and ABA positively regulates rice stomatal immunity against Xoc. Thus, we provide evidence supporting a model in which a major bacterial pathogen inhibits plant stomatal immunity by directly targeting VB6 biosynthesis and consequently inhibiting the biosynthesis of ABA in guard cells to open stomata. Moreover, AvrRxo1-mediated VB6 targeting also explains the poor nutritional quality, including low VB6 levels, of Xoc-infected rice grains.

Key words: rice bacterial leaf streak, effector, stomatal immunity, pyridoxal phosphate synthase, vitamin B6 (VB6), abscisic acid (ABA)

Liu H., Lu C., Li Y., Wu T., Zhang B., Liu B., Feng W., Xu Q., Dong H., He S., Chu Z., and Ding X. (2022). The bacterial effector AvrRxo1 inhibits vitamin B6 biosynthesis to promote infection in rice. Plant Comm. 3, 100324.

INTRODUCTION

Rice leaf streak caused by Xanthomonas oryzae pv. oryzicola (Xoc) is a disease subject to international quarantine that seriously affects rice yield in tropical and subtropical areas of Asia, northern Australia, and parts of West Africa. The incidence and severity of the disease are increasing in parts of Asia where hybrid rice varieties are grown (Nino-Liu et al., 2006). Xoc shares high genomic DNA sequence identity with X. oryzae pv. oryzae (Xoo), which causes rice bacterial blight, but the two pathogen interact with rice in different manners. For example, Xoc invades rice leaves mainly through stomata and colonizes leaf intercellular spaces, whereas Xoo enters rice leaves through hydathodes and multiplies in the xylem. Xoo secretes transcription activator-like effectors through the type three secretion system (T3SS) to induce rice genes, including SWEET-family sugar transporter genes, as part of its pathogenesis mechanism (Yang et al., 2006; Chen et al., 2012). More than 40 resistance (R) genes against Xoo have been identified in rice, whereas rice exhibits no R genes conferring resistance against Xoc strains from Asia (Zhao et al., 2005). The Xoc effector AvrRxo1, which is highly conserved in all tested strains of Xoc, was previously found to suppress nonhost resistance to Xoo in Nicotiana benthamiana (Liu et al., 2014). However, it is unclear whether AvrRxo1 plays a virulence role in its native host plant, rice, owing to the lack of an AvrRxo1-knockout mutant. AvrRxo1 also induces hypersensitive cell death in maize carrying the maize Rxo1 gene (Zhao et al., 2005) and is toxic to bacteria, yeast, and plants (Zhao et al., 2004; Liu et al., 2014; Han et al., 2015; Triplett et al., 2016). Structural analysis has shown that AvrRxo1 contains a T4 polynucleotide kinase domain and is...
AvrRxo1 inhibits vitamin B6 biosynthesis, promoting infection in rice

**RESULTS**

**AvrRxo1 is required for Xoc to reopen the stomata of rice**

Although the *avrRxo1* gene was cloned in 2004 (Zhao et al., 2004), understanding of the virulence function of AvrRxo1 in the compatible Xoc–rice interaction is limited, partly because of the lack of an available *avrRxo1* knockout mutant. We successfully generated an *avrRxo1* knockout mutant designated RS105Δ (Supplemental Figure 1) and evaluated its virulence in Zhonghua11, a japonica-type rice cultivar that is susceptible to *X. oryzae* (*avirulent* strain designated RS105, RS105Δa, or RS105Δa-CP (complementary strain)). Scale bar represents 0.5 cm.

(B) The populations of Xoc strains RS105, RS105Δa, and RS105Δa-CP in the leaves of wild-type Zhonghua11 rice. Bacterial growth was assessed at 14 dpi (n = 6; **P ≤ 0.01; Student’s t-test). (C) Lesion lengths in the leaves of wild-type Zhonghua11 rice vacuum inoculated with Xoc strain RS105, RS105Δa, or RS105Δa-CP at 14 dpi (ns, no significance; Student’s t-test).

(D) Stomatal apertures on the leaves of Zhonghua11 plants exposed to water or Xoc strain RS105, RS105Δa, or RS105Δa-CP at 1 and 8 h after inoculation (n = 20; **P ≤ 0.01; Student’s t-test).

Because Xoc invades unwounded rice leaves mainly through stomata, we measured the stomatal aperture of leaves dip inoculated with RS105 bacterial suspensions. At 1 h post treatment (hpt), the average stomatal aperture on inoculated leaves was significantly smaller than that on water-treated leaves (Figure 1D). At 8 hpt, the stomatal aperture of leaves inoculated with wild-type RS105 or the *avrRxo1*-complementary strain had reverted to the pre-inoculation state. Interestingly, stomatal reopening was not observed in leaves incubated with the RS105Δa mutant (Figure 1D). These results suggest that AvrRxo1 participates in stomatal reopening in rice.

**AvrRxo1 targets the OsPDX1.2 protein in rice**

To elucidate the molecular mechanism by which AvrRxo1 promotes Xoc infection in rice leaves, we performed yeast two-hybrid screening using AvrRxo1 (109–421 amino acid fragments) as bait and a rice cDNA library as prey. We screened five proteins that interacted with AvrRxo1 (Supplemental Table 3), among which OsPDX1.2 (Os10G01080) showed the strongest interaction (Figure 2A). OsPDX1.2 shares high sequence homology with the *Arabidopsis* pyridoxine synthase gene *AtPDX1.3* (*At5G01410*), which is associated with the production of VB6 (Titiz et al., 2006). To confirm whether full-length OsPDX1.2 and AvrRxo1 interact in *vivo*, we performed a bimolecular fluorescence complementation (BiFC) assay and a coimmunoprecipitation (coIP) assay via the transient co-expression of AvrRxo1–MYC-nYFP (N-terminal fragment of yellow fluorescent protein) and OsPDX1.2-hemagglutinin (HA)-cYFP (C-terminal fragment of yellow fluorescent protein) in the leaves of *N. benthamiana*. The yellow fluorescence signal was observed only at the border of the epidermal cells (Figure 2B), suggesting that AvrRxo1 interacts with OsPDX1.2 in plants. The coIP assay results showed that MYC-epitope-tagged AvrRxo1 was coimmunoprecipitated with HA-epitope-tagged OsPDX1.2 (Figure 2C).
AvrRxo1 inhibits vitamin B6 biosynthesis, promoting infection in rice

Figure 2. AvrRxo1 targets and degrades the OsPDX1.2 protein to decrease VB6 levels in rice.

(A) AvrRxo1 interacts with OsPDX1.2 in a yeast two-hybrid assay. The interaction was assessed through the growth of yeast cells on selective medium lacking Leu, Trp, His, and Ade and containing aureobasidin A and X-gal.

(B) AvrRxo1 and OsPDX1.2 interact in plants. AvrRxo1-MYC-nYFP and OsPDX1.2-HA-cYFP were transiently co-expressed in tobacco leaves, and the results were photographed 3 days later. Scale bars represent 20 μm.

(C) AvrRxo1 interacts with OsPDX1.2 in a coIP assay. OsPDX1.2-HA and AvrRxo1-MYC or control (vector alone) were co-expressed in N. benthamiana leaves. coIP was carried out with an anti-MYC antibody, and the proteins were visualized by western blotting with an anti-PDX1 antibody.

(D) AvrRxo1 interacts with OsPDX1.2 in a coIP assay. OsPDX1.2-HA and AvrRxo1-MYC or control (vector alone) were co-expressed in N. benthamiana leaves. coIP was carried out with an anti-MYC antibody, and the proteins were visualized by western blotting with an anti-PDX1 antibody.

(E) OsPDX1.2+AvrRxo1

(F) MBP   AvrRxo1

(G) MP AvrRxo1 K166N T167A D193A

(H) Leaves and Seeds
**Plant Communications**

Deletion analysis indicated that the C termini of AvrRxo1 and PDX1.2 were sufficient for the observed interaction (Supplemental Figure 3). In rice, two other PDX1 proteins, OsPDX1.1 (Os07g01020) and OsPDX1.3 (Os11g48080), share high sequence homology with OsPDX1.2 (93% and 85% identity at the amino acid level, respectively). Both OsPDX1.1 and OsPDX1.3 interact with AvrRxo1 in yeast and plant cells (Supplemental Figure 4). Because previous crystallization studies showed that PDX1 proteins from Bacillus subtilis form a synthase complex (Mooney et al., 2009), we analyzed the interactions among PDX1 proteins and found that PDX1 proteins from rice can assemble into homo- and heterodimers (Supplemental Figure 5). We also tested the interaction of AvrRxo1 with PDX1 proteins from the nonhost plant Arabidopsis and with SNZ1, a homolog involved in pyridoxine biosynthesis from yeast. All three PDX1 proteins (AtPDX1.1, AtPDX1.2, and AtPDX1.3) of Arabidopsis and SNZ1 of yeast interacted with AvrRxo1 (Supplemental Figure 4). Together, these data suggest that interaction with AvrRxo1 is a common feature of the PDX1 protein family that is conserved in rice (a monocot), Arabidopsis (a dicot), and yeast (a fungus), consistent with previous observations of the common toxic activity of AvrRxo1 in several plants and yeasts (Liu et al., 2014; Triplett et al., 2016).

**AvrRxo1 targets and degrades OsPDX1.2 protein in rice**

The AvrRxo1 protein is predicted to contain a protease motif (Zhao et al., 2004), which may affect the stability of PDX1 proteins. We therefore monitored the levels of the OsPDX1.2 protein in rice after Xoc infection. Compared with control rice plants treated with H2O, the leaves of rice plants sprayed with RS105 or the complementary strain RS105Δa-CP showed a significantly decreased level of the OsPDX1.2 protein (Figure 2D). By contrast, rice leaves treated with RS105Δa showed a similar level of OsPDX1.2 to that observed in the H2O-treated control. These results showed that AvrRxo1 is required for Xoc to decrease the level of OsPDX1.2 during infection. We also examined the levels of AtPDX1 proteins in dexamethasone-inducible, avrRxo1-expressing transgenic Arabidopsis plants (Col-0 genetic background). The dexamethasone-induced expression of AvrRxo1 resulted in a significant reduction in the AtPDX1 protein level at 24 hpt (Supplemental Figure 7).

The reduction in OsPDX1.2 and AtPDX1 levels in an AvrRxo1-dependent manner observed in vivo raised the possibility that AvrRxo1 may directly act as a protease on PDX1 proteins. To test this hypothesis, we performed enzyme catalysis assays using purified AvrRxo1 and OsPDX1.2 proteins and detected the degradation of OsPDX1.2 within 15 min upon AvrRxo1 addition. With increased reaction times, more OsPDX1.2 was degraded (Figure 2E). In addition to the presence of a putative protease domain, AvrRxo1 contains a putative ATP-binding motif (Zhao et al., 2004), raising the possibility that AvrRxo1 may be an ATP-dependent protease. We therefore carried out a protein degradation assay in the presence of different concentrations of ATP. In reactions containing 1 or 10 mM ATP, OsPDX1.2 proteins were degraded by AvrRxo1, whereas in reactions containing less than 0.1 mM ATP, AvrRxo1 failed to degrade OsPDX1.2 (Figure 2F). We also generated AvrRxo1K166N and AvrRxo1T167A, which harbored mutations in the ATP-binding motif, and examined their protease activity. We found that the AvrRxo1K166N and AvrRxo1T167A mutants failed to degrade OsPDX1.2 (Figure 2G). These results indicate that ATP is required for AvrRxo1 to degrade OsPDX1.2 proteins. In addition, we examined the protease activity of the AvrRxo1D193A mutant, with a mutation at the putative kinase catalytic aspartic acid residue D193 (Han et al., 2015; Shidore et al., 2017). We observed that AvrRxo1D193A could still degrade OsPDX1.2 proteins (Figure 2G), suggesting that this putative kinase activity is not required for the protease activity of AvrRxo1. The results above reveal that AvrRxo1 targets and degrades OsPDX1.2 protein in rice infected by Xoc.

**AvrRxo1 decreases the level of VB6 in leaves and seeds of rice**

Because OsPDX1.1 and OsPDX1.2 are involved in VB6 biosynthesis in rice (Chen et al., 2014), we next investigated whether AvrRxo1 modulates the level of VB6 during Xoc infection by examining rice leaves inoculated with the RS105 or RS105Δa strain. Compared with plants subjected to the control (H2O) treatment, plants inoculated with RS105 showed significantly lower VB6 levels in their leaves (Figure 2H). Interestingly, the VB6 levels of RS105Δa mutant-inoculated plants were significantly higher than those of RS105-inoculated plants but were lower than those of control-treated plants (Figure 2H), suggesting that AvrRxo1 is likely to be one of the virulence factors involved in reducing VB6 levels. We also examined the levels of VB6 in rice seeds after RS105 or RS105Δa inoculation. A lower level of VB6 was observed in the seeds of rice inoculated with RS105 than in those of the control (Figure 2H), suggesting that Xoc invasion could decrease the nutritional content of rice.

**OsPDX1 and VB6 positively regulate rice immunity to Xoc**

Our results obtained thus far suggested that, during Xoc invasion, AvrRxo1 targets OsPDX1 proteins to inhibit the synthesis of VB6. VB6 has been shown to play important roles in plant growth, development, and abiotic stress responses (Mooney et al., 2009), and it...
Arabidopsis (Supplemental Figure 11). Interestingly, VB6-induced stomata closure in both rice (Figure 4C) and rice leaves treated with VB6. We found that VB6 treatment caused the stomata to close in both rice (Figure 4C) and Arabidopsis (Supplemental Figure 11). Interestingly, VB6-induced stomatal closure is dependent on ABA biosynthesis and signaling. Specifically, compared with the results observed in wild-type rice plants, stomatal responses to VB6 or ABA were greatly compromised in the leaves of the ABA-insensitive mutant osbzip23 (Figure 4C). In Arabidopsis, the VB6-triggered stomatal responses of the ABA biosynthetic mutant aba2-1 and the ABA-signaling mutant abi1-1 were compromised (Supplemental Figure 11).

VB6 acts as a cofactor of numerous enzymes, such as those catalyzing the metabolism of amino acids and fatty acids (Mooney et al., 2009). We suspected that VB6 might promote the biosynthesis of ABA in plants. To test this hypothesis, we examined the levels of ABA in plants treated with VB6. The high-performance liquid chromatography (HPLC)-mass spectrometry (MS/MS) assay showed that the level of ABA was greatly increased after VB6 treatment (Figure 4D). VB6-activated ABA biosynthesis could also be visualized via an immunohistochemistry assay using an ABA-specific antibody. Enhanced immunofluorescence was detected in guard cells of rice leaves treated with VB6 (Figure 4E). Similarly, VB6 was found to promote ABA biosynthesis in Arabidopsis leaves (Supplemental Figure 12). These results strongly suggest that VB6 activates ABA biosynthesis as a mechanism to promote stomatal closure.

Because Xoc invasion reduces the level of VB6 (Figure 2H) and VB6 activates ABA biosynthesis (Figure 4D and 4E), we hypothesized that Xoc invasion leads to a reduction in ABA levels as a virulence strategy. To test this hypothesis, we quantified ABA concentrations in rice plants inoculated with the RS105, RS105Δα, or RS105Δα-complemented bacterial suspension. The results of both the HPLC-MS/MS assay (Figure 4F) and the immunohistochemistry assay (Figure 4G and Supplemental Figure 13) showed that the levels of ABA in the leaves of rice infected with RS105 or RS105Δα-complemented strains were significantly lower than those in H2O-treated and RS105Δα-inoculated plants.

VB6 is required as a cofactor for the biosynthesis of ABA
We next investigated how VB6 promotes ABA biosynthesis. VB6 is required for the activation of molybdenum cofactor (Moco) by Moco sulfurtransferase (Seo et al., 2000a). In plants, aldehyde oxidase 3 (AO3) requires activated Moco to catalyze the oxidation of abscisic aldehyde to generate ABA (Seo et al., 2000b; Bittner et al., 2001). We therefore tested the activity of aldehyde oxidase in OsPDX1.2-OE transgenic plants and OsPDX1-KO plants by native polyacrylamide gel electrophoresis. As shown in Figure 4H, a significant decrease in AO3 activity was detected in the leaves of OsPDX1-KO plants, whereas in OsPDX1.2-OE transgenic plants, AO3 exhibited an intense band corresponding to ABA-aldehyde. We also detected a marked reduction in aldehyde oxidase activity in Xoc-inoculated plants (Figure 4I). Taken together, these results suggest that VB6 is required for aldehyde oxidase activity in ABA biosynthesis in plants.

ABA positively regulates rice immunity to Xoc
Because VB6 is required for ABA biosynthesis and positively regulates the stomatal immunity of rice against Xoc, we tested whether ABA affects rice defense against Xoc. We found that ABA treatment significantly reduced bacterial multiplication in the leaves of rice (Figure 5A). Conversely, the ABA-deficient mutant osaba1 and the ABA-insensitive mutant bzip23 showed significantly increased susceptibility to Xoc multiplication (Figure 5B and 5C). In addition, transgenic rice lines overexpressing OsABA3 were generated and showed enhanced resistance to Xoc (Figure 5D). These results suggest that ABA also positively regulates rice immunity to Xoc.
Figure 3. OsPDX1 and VB6 positively regulate rice stomatal immunity against Xoc.
(A) The populations of Xoc strain RS105 in the leaves of wild-type Zhonghua11 rice or OsPDX1-RNAi T1 generation transgenic plants. Bacterial growth was assessed at 14 dpi (n ≥ 6; **P ≤ 0.01; Student’s t-test).
(B) The populations of Xoc strain RS105 in the leaves of wild-type Zhonghua11 rice or OsPDX1.2-overexpressing T1 generation transgenic plants. Bacterial growth was assessed at 14 dpi (n ≥ 6; **P ≤ 0.01; Student’s t-test).
(C) VB6 levels in the leaves of wild-type Zhonghua11 rice, OsPDX1-RNAi, OsPDX1.2-overexpressing T1 generation transgenic, or OsPDX1.2-knockout mutant plants (mean ± SD; n ≥ 5; **P ≤ 0.01; Student’s t-test).
(D) The populations of Xoc strain RS105 in the leaves of wild-type Zhonghua11 rice or OsPDX1.2-knockout mutant plants. Bacterial growth was assessed at 14 dpi (n ≥ 6; **P ≤ 0.01; Student’s t-test).
(E) VB6 enhances rice defense against Xoc. Populations of Xoc strain RS105 in the leaves of wild-type Zhonghua11 plants pretreated with the control treatment or different concentrations of VB6. Bacterial growth was assessed at 14 dpi (n ≥ 6; **P ≤ 0.01; Student’s t-test).
(F) Stomatal apertures on the leaves of wild-type Zhonghua11, OsPDX1.2-knockout mutant plants, or OsPDX1.2-overexpressing transgenic plants exposed to water or Xoc strain RS105, RS105Δα, or RS105Δα-CP at 8 h after inoculation (n ≥ 23; **P ≤ 0.01; Student’s t-test).
Figure 4. VB6 promotes ABA biosynthesis and stomatal closure in rice.
(A) OsPDX1.2 is associated with ABA metabolism in rice. The ABA levels in the leaves of wild-type Zhonghua11, OsPDX1.2-knockout mutant, or OsPDX1.2-overexpressing rice were measured by HPLC–MS/MS (mean ± SD; n = 3; **P < 0.01; Student’s t-test).
(B) The distribution of ABA accumulation, as measured by the optimized immunofluorescence technique, in the leaves of wild-type Zhonghua11, OsPDX1.2-knockout mutant, or OsPDX1.2-overexpressing rice. The ABA/Alexa Fluor 555 fluorescence intensity analyzed by ImageJ was taken as the ABA concentration index (n = 20; **P < 0.01; Student’s t-test).
(C) Stomatal apertures on the leaves of wild-type Zhonghua11 or bzip23 (ABA-insensitive mutant) plants exposed to water, VB6, or ABA (n ≥ 12; **P < 0.01; Student’s t-test).
(D) ABA levels in the leaves of wild-type Zhonghua11 plants treated with water or VB6 (n ≥ 3; **P < 0.01; Student’s t-test). The leaves were harvested at 4 h posttreatment.
(E) The distribution of ABA accumulation in the leaves of wild-type Zhonghua11 (n ≥ 20; **P < 0.01; Student’s t-test). Leaves were harvested at 8 h after water or VB6 treatment. Scale bars represent 1 μm. The ABA concentration index was examined as indicated in (B).
Plant Communications

AvrRxo1 inhibits vitamin B6 biosynthesis, promoting infection in rice

DISCUSSION

In this research, we found that AvrRxo1 targets and degrades OsPDX1.2, which is involved in the biosynthesis of VB6, leading to a decreased ABA level and the reopening of closed stomata (Figure 6). Previous studies showed that AvrRxo1 can phosphorylate NAD to generate 3'-NADP in bacteria, yeast, and plants (Schuebel et al., 2016; Triplet et al., 2016; Shidore et al., 2017). However, this activity could not clearly explain the virulence function of AvrRxo1 revealed in our avrRxo1 mutant analysis. For example, reduced NAD is expected to increase ABA sensitivity (Hong et al., 2020), which conflicts with the role of ABA in regulating stomatal closure. In addition, a previous study showed that NAD kinase activity is not always associated with the toxicity of AvrRxo1 or the suppression of reactive oxygen species (ROS) by AvrRxo1 (Shidore et al., 2017). AvrRxo1-mediated NAD phosphorylation to produce 3'-NADP may play some role in AvrRxo1 toxicity, but protease activity is required for its virulence function in the context of Xoc infection in rice leaves, as demonstrated in this study.

ABA plays complex roles in the regulation of plant–microbe interactions (Lievens et al., 2017). As a key regulator of stomatal closure, ABA positively regulates immunity to pathogens that enter host plants mainly through the stomata, but some reports have found that ABA enhances plant susceptibility to certain pathogens (Liao et al., 2018; Zhang et al., 2019). In Arabidopsis, the ABA-deficient aba3-1 mutant exhibits compromised microbe-associated molecular pattern (MAMP)- and bacterium-induced stomatal closure (Melotto et al., 2006). Similarly, Pst DC3000 does not induce stomatal closure in the notabilis tomato mutant, which lacks the ABA synthesis gene NCED 1 (Du et al., 2014). OPEN STOMATA1 is one of the core signaling components of the ABA pathway, and the os1-2 mutant is defective in MAMP- and bacterium-induced stomatal closure (Melotto et al., 2006). However, some studies have shown that chitosan and the yeast elicitor YEL are able to induce stomatal closure in the aba2-2 mutant and ABA-biosynthesis-inhibitor-treated plants (Issak et al., 2013). It has also been reported that the ABA-independent oxylipin pathway controls stomatal closure and immune defense in Arabidopsis (Montillet et al., 2013). In addition, a recent study demonstrated that fig22-induced stomatal closure was associated with the phosphorylation of OSCA1.3, a Ca2+-permeable channel that does not regulate stomatal closure upon the perception of ABA (Thor et al., 2020). These results suggest that both ABA-dependent and ABA-independent pathways play roles in stomatal defense. In this work, we found that VB6 induces stomatal closure by promoting the biosynthesis of ABA and that ABA biosynthesis and signaling are required for VB6-induced stomatal closure (Figure 4 and Supplemental Figure 11). VB6 acts as a cofactor of ABA3, which can convert Moco from the desulfo-form into the sulfo-form. The sulfo-form of Moco is required for aldehyde oxidase AAO3 to catalyze the transformation of abscisic aldehyde to ABA (Seo et al., 2000b; Bittner et al., 2001). Thus, the AvrRxo1-mediated inhibition of VB6 biosynthesis revealed a previously unknown step in ABA biosynthesis in plants, in addition to illuminating a novel mechanism of stomatal immunity suppression by a pathogen.

In plants, stomatal closure to prevent bacteria from entering leaves has emerged as a conserved mechanism in the activation of innate immunity. Stomatal defense was first discovered while studying the Arabidopsis–Pst DC3000 interaction and was subsequently found in other plants, such as tomato and tobacco (Du et al., 2014; Murray et al., 2016). Until now, however, little has been known about whether monocots also employ stomatal defense against pathogenic bacteria. Our results show that Xoc invasion induces rapid stomatal closure within 1 h (Figure 1D), suggesting that stomatal defense is also involved in rice–bacteria interactions. To counteract stomatal immunity, other bacteria secrete phytoxins or T3SS effectors to overcome stomatal defense (Melotto et al., 2017). In Arabidopsis, some Pst DC3000 effectors, such as HopF2, reduce the MAMP-triggered ROS burst to inhibit stomatal closure; other effectors, such as HopX1, HopZ1, AvrB, and the phytoxins COR, induce jasmonic acid signaling, and the proteasome inhibitor syringolin A suppresses the salicylic acid (SA) response to inhibit stomatal defense (Schellenberg et al., 2010; Xin and He, 2013; Zheng et al., 2015; Gimenez-Ibáñez et al., 2017). Recently, it was reported that the T3SS effector XopC2 of Xoc, as a protein kinase, phosphorylates the key component of SCFOrsC011b to promote JAZ degradation and jasmonic acid signaling, which results in stomatal reopening (Wang et al., 2021). Here, we identified the VB6 biosynthesis gene OsPDX1.2 as a new target of bacterial effectors to overcome stomatal defense. Notably, in addition to its presence in all Xoc strains, avrRxo1 is widely conserved in other bacterial pathogens, such as Acidovorax and Burkholderia, which infect diverse plants. It remains to be determined whether AvrRxo1 targets PDX1 proteins in these plant–pathogen interactions as a widespread mechanism facilitating bacterial invasion.

PDX1 is the major gene of de novo VB6 biosynthesis that catalyzes more than 400 enzymes and plays an essential role in plant growth and development (Titiz et al., 2006). Because of its capacity to quench ROS, VB6 has also been found to regulate plant response to biotic and abiotic stresses. The expression of Arabidopsis PDX1 genes is regulated by abiotic stresses like high light, drought, chilling, ozone, and UV radiation (Denslow et al., 2007), and the Arabidopsis pxd1.3 mutant is hypersensitive to salt and UV-B treatments (Titiz et al., 2010). In
AvrRxo1 inhibits vitamin B6 biosynthesis, promoting infection in rice

Figure 5. ABA positively regulates rice immunity to Xoc.
(A) ABA enhances rice defense against Xoc. The populations of Xoc strain RS105 in the leaves of wild-type Zhonghua11 plants pretreated with the control treatment or ABA (1 μM) are shown. Bacterial growth was assessed at 14 dpi (n ≥ 6; *P ≤ 0.01; Student’s t-test).
(B) The rice osaba1 mutant is more susceptible to Xoc. The populations of Xoc strain RS105 in the leaves of wild-type Nipponbare rice or osaba1 mutant plants were assessed at 14 dpi (n = 18; **P ≤ 0.01; Student’s t-test).
(C) The ABA-insensitive mutant bzip23 is more susceptible to Xoc. The populations of Xoc strain RS105 in the leaves of wild-type Zhonghua11 rice or bzip23 mutant plants were assessed at 14 dpi (n ≥ 6; *P ≤ 0.01; Student’s t-test).
(D) OsABA3-overexpressing T1 generation transgenic plants were more resistant to Xoc. The populations of Xoc strain RS105 in the leaves of wild-type Zhonghua11 rice or OsABA3-overexpressing plants were assessed at 14 dpi (n = 6; **P ≤ 0.01; Student’s t-test).

METHODS

Bacterial strains and plasmid construction

The bacterial strains and plasmids used in this study are described in Supplemental Table 1. The Escherichia coli strains were cultured on Luria-Bertani medium at 37°C. The Xoc RS105 strain was cultured on nutrient agar (NA) medium containing 50 μg ml⁻¹ rifampicin at 28°C. The Pseudomonas syringae pv. tomato strain DC3000 was cultured on King’s B medium containing 50 μg ml⁻¹ rifampicin at 28°C. The Agrobacterium tumefaciens strains were cultured on Luria-Bertani medium containing 50 μg ml⁻¹ rifampicin at 28°C.

To generate the avrRxo1 KO mutant, a 430-bp fragment located 88–517 bp downstream of the ATG start codon was amplified using the primers avrRxo1KO-F and avrRxo1KO-R. The fragment was inserted into the pEASY-T1 vector (TransGen, China), the construct was transformed into E. coli strain BL-21 by electrotransformation. To generate pHM1-avrRxo1, the avrRxo1 gene with its promoter was amplified using the primers AvrRxo1-ORF1-F and AvrRxo1-ORF2-R, which contained Sac I sequences at both the 5’ and 3’ ends, followed by Sac I digestion and ligation into the pHM1 vector (Yang et al., 2006). pHM1-avrRxo1 was transformed into RS105Δavr (avrRxo1 KO mutant) by electroporation and cultured on NA medium containing 50 μg ml⁻¹ rifampicin, 50 μg ml⁻¹ spectinomycin, and 50 μg ml⁻¹ kanamycin at 28°C.

In a yeast two-hybrid system, avrRxo1(109–421), avrRxo1(160–421), avrRxo1(164–421), avrRxo1(194–421), avrRxo1(230–421), avrRxo1(309–421), avrRxo1(364–421), avrRxo1(1–411), OsPDX1.1, OsPDX1.2, OsPDX1.2(1–313), OsPDX1.2(157–313), OsPDX1.2(184–313), OsPDX1.2(199–313), OsPDX1.2(246–313), OsPDX1.2(283–313), OsPDX1.2(273–313), OsPDX1.2(263–313), and OsPDX1.2(253–263) were amplified using the primers containing Nco I and BamHI sites listed in Supplemental Table 2 and inserted into the pGBK7 or pGADT7 (Clontech) vector. The plasmids were transformed into Y2H Gold cells and plated on double dropout medium (SD-Leu/-Trp) and quadruple dropout medium (SD-His/-Leu/-Trp/-Ura) (Clontech).

For BiFC and coIP assays, the AvrRxo1 and OsPDX1.2 genes were amplified by PCR using primers containing BamHI and Hind III sites, and the products were cloned into the pSPYNE and pSPYCE vectors (Valter et al., 2004), respectively. OsPDX1.1-cYFP and OsPDX1.3-cYFP were generated using a similar method. The primers used to amplify avrRxo1, OsPDX1.1, OsPDX1.2, and OsPDX1.3 are described in Supplemental Table 2.

AvrRxo1 fused with MBP and 6xHis tag was used for Escherichia coli fusion protein isolation. The AvrRxo1 gene was amplified using primers containing BamHI and Hind III digestion and ligation into the pMal-c2x vector (NEB). The AvrRxo1 point mutants AvrRxo1K166N, AvrRxo1T167A, and AvrRxo1D193A were generated by site-directed mutagenesis with the primers listed in Supplemental Table 2 using the pSPYNE and pSPYCE vectors (Walter et al., 2004), respectively. OsPDX1.1-cYFP and OsPDX1.3-cYFP were used as a control. The OsPDX1.2 gene was cloned into the pDONR201 entry vector via the BP reaction and then subcloned into the pET-DEST42 vector (Invitrogen). These constructs were transformed into E. coli strain BL-21 by electrotransformation.

The plasmids Pab:OsPDX1.1, Pab:OsPDX1.2, Pab:OsPDX1.3, and Pab:OsABA3 were constructed by introducing the full-length OsPDX1.1, OsPDX1.2, OsPDX1.3, and OsABA3 sequences into the Xcm I-digested plasmid pCXUN-HA (Chen et al., 2009). cDNA from Zhonghua11 was used as a template to amplify these fragments.

To silence OsPDX1.2 in rice, a 263-bp fragment located at the 3’ end of the OsPDX1.2 gene was amplified using Zhonghua11 genomic DNA as a
template, and the PCR products were digested with KpnI/BamHI and inserted into the plasmid pDS1301 to obtain pDS1301-OsPDX1.2. Then, the pDS1301-OsPDX1.2 and OsPDX1.2 fragments were digested with SacI/SpeI and incubated with T4 DNA ligase (NEB) to obtain OsPDX1.2-RNAi.

A CRISPR–Cas9 system obtained from Yaoguang Liu was used to knock out OsPDX1.2. The plasmids used for this purpose were constructed as described previously (Ma et al., 2016). The target sequence was 5ʹ-GTCATGGCCAAGGCCCGCAT-3ʹ, which is located 286–305 bp downstream of the ATG start codon. All plasmids were validated by sequencing.

**Plant material and growth conditions**

Rice (*Oryza sativa* japonica) plants of the wild-type Zhonghua11 and Nipponbare; the *bzip23* (Zhonghua11 background; Xiang et al., 2008), *osaba1* (Nipponbare background), and *OsPDX1.2* KO mutants; and the *OsPDX1.2* -OX, *OsPDX1.2* -RNAi, and *OsABA3* -OX transgenic lines were grown in soil in a growth house with 12-h days (at 28°C and 60%–75% relative humidity) and 12-h nights (at 24°C and 60%–75% relative humidity).

*Arabidopsis thaliana* wild-type Col-0, Ler, *abi1-1* (Leung et al., 1994), and transgenic lines containing DEX:AvrRxo1 were grown in nutrient substrates in an incubator with 12-h days (at 22°C and 60%–75% relative humidity) and 12-h nights (at 20°C and 60%–75% relative humidity).

*N. benthamiana* plants were grown in a greenhouse with 12-h days (at 22°C and 60%–75% relative humidity) and 12-h nights (at 20°C and 60%–75% relative humidity).

**Rice and *Arabidopsis* transformation**

Transgenic rice plants were constructed via *Agrobacterium*-mediated transformation according to published protocols (Lin and Zhang, 2005). *Agrobacterium*-mediated *Arabidopsis* transformation was performed by floral dipping as described previously (Clough and Bent, 1998).

A CRISPR-Cas9 system obtained from Yaoguang Liu was used to knock out OsPDX1.2. The primers used to sequence the target OsPDX1 are listed in Supplemental Table 2.

**Chemical treatments**

To study the effects of VB6 or ABA on disease development, VB6 (Sigma-Aldrich) or ABA (Sigma-Aldrich) solubilized in deionized H2O supplemented with 0.05% (vol/vol) Tween 20 or ddH2O (as a control) was sprayed on rice plants, and 4 h later, the rice plants were sprayed with bacterial suspensions (10⁸ colony-forming units [CFU] ml⁻¹) again at 4 and 8 days after inoculation. Bacterial growth was measured at 14 days after inoculation.

**Xoc infection and bacterial growth assay**

To evaluate bacterial leaf streak disease, 4-week-old rice plants were inoculated with bacterial suspensions (10⁶ CFU ml⁻¹) by spray or infiltration. For the spray assay, the rice was completely sprayed with the bacterial suspension in buffer containing 10 mM MgCl₂ supplemented with 0.05% (vol/vol) Tween 20 or ddH₂O (as a control) was sprayed on rice plants, and 4 h later, the rice plants were sprayed with bacterial suspensions (10⁸ colony-forming units [CFU] ml⁻¹). The plants were sprayed with VB6, ABA, or ddH₂O again at 4 and 8 days after inoculation. Bacterial growth was measured at 14 days after inoculation.

To analyze the effects of Xoc infection on the VB6 level in seeds, rice plants at the bolting stage were completely sprayed with bacterial suspension in buffer containing 10 mM MgCl₂ supplemented with 0.05% (vol/vol) Tween 20. For the infiltration assay, the bacterial suspension in buffer containing 10 mM MgCl₂ was infiltrated into rice leaves with a needleless syringe. Bacterial growth was measured at 14 days after inoculation.

A bacterial growth assay was performed in rice plants at 14 days after inoculation. Ten-centimeter-long leaves from six independent plants were infected with *Xanthomonas oryzae* pv. *oryzae* (Xoc) strain 6705 at 14 days after inoculation.
AvrRxo1 inhibits vitamin B6 biosynthesis, promoting infection in rice

were harvested, surface sterilized in a 70% ethanol solution for 1 min, and then rinsed in sterile distilled water three times. The samples were ground in 2 ml of sterile distilled water followed by serial dilution (1:10) and plating on NA medium containing 50 μg ml⁻¹ rifampicin at 28°C for colony counting. The experiment was repeated three times. The means were compared using Student’s t-test. The standard error and t-test results were recorded.

Stomatal aperture measurement

The stomatal apertures on the leaves of rice or Arabidopsis plants were measured as reported by Melotto et al. (2006). Rice or Arabidopsis plants were kept under light for at least 3 h and then treated with VB6 or ABA or inoculated with Xoc strains. Leaves were harvested at 1 and 8 h after treatment. The stomatal apertures were measured using an LSM 880 NLO confocal microscope (Zeiss).

RNA extraction, reverse transcription-polymerase chain reaction, and quantitative real-time PCR

Leaves or whole plants were collected to extract total RNA using the TRIzol Reagent (Invitrogen). ReverTra Ace qPCR RT Master Mix with gDNA Remover (TOYOBO) and 1 μg of total RNA were used to synthesize first-strand cDNA according to the manufacturer’s instructions. Quantitative real-time PCR was performed with a QuantStudio 6 Flex Real-Time PCR System (Life Technologies) using KOD SYBR qPCR Mix (TOYOBO) as described in the manufacturer’s instructions. The primers used for quantitative real-time PCR are described in Supplemental Table 2.

Protein interaction analysis

Y2H assays were used to screen protein interactions with the Matchmaker Gold Yeast Two-Hybrid System (Clontech) as described in the manufacturer’s instructions. In brief, the plasmids pGBKTT7-avrRxo1(109–421) and pGADT7-OsPDX1.2 were transformed into Y2H Gold yeast cells, which were then grown on double dropout medium (SD-Leu/-Trp) and quadruple dropout medium (SD-His/-Leu/-Trp/-Ura).

BIFC was used to examine the protein interactions as described previously (Walter et al., 2004). In brief, Agrobacterium GV3101 cells containing the AvrRxo1-MYC-nYFP and OsPDX1.2-HA-cYFP plasmids were resuspended in agroinfiltration buffer (10 mM MgCl₂, 10 mM 2-(N-morpholin)ethanesulfonic acid (MES), pH 5.6, 200 mM acetoxyringsone), and the optical density at 600 nm was adjusted to 1.0. These suspensions were mixed in equal amounts and infiltrated into 4-week-old N. benthamiana leaves with a needleless syringe. Three days later, the leaves were observed using an LSM 880 NLO confocal microscope (Zeiss).

A coIP assay was performed to examine protein interactions as described previously (Moffett et al., 2002). In brief, the agroinfiltrated tobacco leaves were harvested and ground to a fine powder in liquid nitrogen, and the powder was resuspended in protein extraction buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 50 mM glucose, 0.1% Triton X-100, 0.5 mM dithiothreitol, 10 mM NaF, 1 mM Na₃VO₄, 25 mM β-sodium glycerophosphate, 1 mM PMSF, and 1X protease inhibitor cocktail [Sigma]) for 1 h. MYC-Trap beads were used to imnunoprecipitate the proteins, and the eluted proteins were further analyzed by SDS–PAGE and western blotting. An anti-OsPDX1 antibody raised against DDAHHINKHN epitope peptides was produced by Abmart and used at a 1:1000 dilution. The anti-c-Myc tag antibody was used against c-Myc-tagged protein (1:3000; Abmart). The secondary antibody used was horseradish-peroxidase-conjugated goat anti-mouse immunoglobulin G (Sigma; 1:10 000).

Protein purification and degradation analyses

The MBP-AvrRxo1-6×His and OsPDX1.2-6×His proteins were purified by Ni⁺⁺ affinity chromatography using cComplete His-Tag Purification Resin (Roche). Protein concentrations were measured with a NanoDrop spectrophotometer and via SDS–PAGE. The purified MBP-AvrRxo1-6×His protein (1 μg) and OsPDX1.2-6×His proteins (5 μg) were mixed in reaction buffer (25 mM Tris-HCl [pH 7.5], 10 mM MgCl₂, 10 mM NaCl, 10 mM ATP) and incubated at 37°C for 2 h. The interaction products were further analyzed by SDS–PAGE and western blotting.

Quantitation of VB6

VB6 was extracted and quantitated as described previously (Wagner et al., 2009). In brief, leaves of 4-week-old rice plants and ripe seeds were powdered in liquid nitrogen, suspended in 0.1 M HCl, and boiled for 30 min at 120°C. The extracts were treated with acid phosphatase (Sigma-Aldrich) and β-glucosidase (Sigma-Aldrich). The contents of pyridoxine, pyridoxal, and pyridoxamine were determined via HPLC using solvents A (0.1% acetic acid) and B (acetonitrile). Pyridoxine–hydrochloride, pyridoxal-hydrochloride, and PM dihydrochloride (Sigma-Aldrich) served as standards.

Quantitation of ABA levels

The leaves of 4-week-old seedlings were used for ABA quantification. To examine the effects of Xoc invasion on the level of ABA, rice seedlings were dip inoculated with RS105, RS105Δa (avrRxo1 KO mutant), or RS105Δa-CP (the complementary strain). To examine the effects of VB6 on the level of ABA, leaves of 4-week-old seedlings were treated with VB6 (100 μM) or ddH₂O by injection. Samples of 100 mg each were prepared and quantified using an HPLC–MS/MS system as reported previously (Xu et al., 2016). At least three biological replicates were analyzed. ABA was also detected using immunocytochemistry in the leaves of rice and Arabidopsis as reported elsewhere (Ondzghi-Assoume et al., 2016). In brief, the leaves were soaked in buffer, vacuum infiltrated at 4°C, and then incubated at 4°C overnight with shaking. Thereafter, the samples were hyalinized and incubated with an anti-ABA polyclonal antibody (Invitrogen). Next, the samples were incubated with a goat anti-rabbit immunoglobulin G secondary antibody (Invitrogen). The resulting fluorescence signals were measured using an LSM 880 NLO confocal microscope (Zeiss).

Aldehyde oxidase activity assay

Aldehyde oxidase activity was analyzed as reported previously (Seo et al., 2000a). Rice leaves were ground to a powder in liquid nitrogen and suspended in excitation buffer (50 mM Tris-HCl [pH 7.5], 1 mM EDTA, 1 μM sodium molybdate, 10 μM flavin adenine dinucleotide, 2 mM dithiothreitol) to extract total proteins, which were then subjected to native PAGE. The bands corresponding to absciscic aldehyde activity were developed with abscisic aldehyde (Sigma-Aldrich) at 30°C in the dark for 30 min.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at Plant Communications Online.

FUNDING

This study was supported by the National Natural Science Foundation (31872925 and 32072500), Natural Science Outstanding Youth Fund of Shandong Province (JQ201807), Shandong Province Key Research and Development Plan (2019JZZY020608, 2020CXKC010803, and 2019GN C106152), Science and Technology Support Plan for Youth Innovation of Colleges and Universities of Shandong Province (2019KJF023), and the National Key Research and Development Program of China (2016YFD0100903). X.D. thanks S.H. for hosting his research visit at Michigan State University, supported by the United States National Institute of General Medical Sciences (GM109928).

AUTHOR CONTRIBUTIONS

X.D., H.L., and Z.C. conceived and designed the experiments. H.L., C.L., T.W., B.Z., B.L., Q.X., and W.F. performed the experiments. H.L., S.H., Y.L., and X.D. analyzed the data. H.L., S.H., and X.D. wrote the manuscript, and H.D. helped with revisions.
AvrRxo1 inhibits vitamin B6 biosynthesis, promoting infection in rice

Leung, J., Bouvier-Durand, M., Morris, P.C., Guerrier, D., Chefdor, F., and Giraudat, J. (1994). Arabidopsis ABA response gene AB1: features of a calcium-modulated protein phosphatase. Science 264:1448–1452.

Liao, Y., Bai, Q., Xu, P., Wu, T., Guo, D., Peng, Y., Zhang, H., Deng, X., Chen, X., Luo, M., et al. (2018). Mutation in rice Abscisic Acid2 results in cell death, enhanced disease-resistance, altered seed dormancy and development. Front. Plant Sci. 9:405.

Lievens, L., Pollier, J., Goossens, A., et al. (2017). Abscisic acid as pathogen effector and immune regulator. Front. Plant Sci. 8:587.

Lim, C.W., and Lee, S.C. (2015). Arabidopsis abscisic acid receptors play an important role in disease resistance. Plant Mol. Biol. 88:313–324.

Lin, Y.J., and Zhang, Q.F. (2005). Optimising the tissue culture conditions for high efficiency transformation of indica rice. Plant Cell Rep. 23:540–547.

Liu, H., Chang, Q., Feng, W., Zhang, B., Wu, T., Li, N., Yao, F., Ding, X., and Chu, Z. (2014). Domain dissection of avrRxO1 for suppressor, avirulence and cytotoxicity functions. PLoS One 9:e113875.

Ma, X., Zhu, Q., Chen, Y., and Liu, Y.G. (2016). CRISPR/Cas9 platforms for genome editing in plants: developments and applications. Mol. Plant. 9:961–974.

Melotto, M., Underwood, W., Koczkan, J., Nomura, K., and He, S.Y. (2006). Plant stomata function in innate immunity against bacterial infection. Cell 126:969–980.

Melotto, M., Zhang, L., Olesssuc, P.R., and He, S.Y. (2017). Stomatal defense a decade later. Plant Physiol. 174:561–571.

Moffett, P., Farnham, G., Peart, J., and Baulcombe, D.C. (2002). Interaction between domains of a plant NBS-LRR protein in disease resistance-related cell death. EMBO J. 21:4511–4519.

Montillet, J., Leonhardt, N., Mondy, S., Tranchimand, S., Rumeau, D., Boudsocq, M., Garcia, A.V., Douki, T., Bigeard, J., Laurière, C., et al. (2013). An abscisic acid–independent oxylipin pathway controls stomatal closure and immune defense in Arabidopsis. Plos Biol. 11:e1001513.

Mooney, S., Leuendorf, J., Hendrickson, C., and Hellmann, H. (2009). Vitamin B6: a long known compound of surprising complexity. Molecules 14:329–351.

Murray, R.R., Emblow, M.S.M., Hetherington, A.M., and Foster, G.D. (2016). Plant virus infections control stomatal development. Sci. Rep. 6:34507.

Nino-Liu, D., Ronald, P., and Bogdanove, A.J. (2006). Xanthomonas oryzae pathogens: model pathogens of a model crop. Mol. Plant Pathol. 7:303–324.

Onozhigli-Assoume, C.A., Chakraborty, S., and Harris, J.M. (2016). Environmental nitrate stimulates abscisic acid accumulation in Arabidopsis root tips by releasing it from inactive stores. Plant Cell 28:729–745.

Schellenberg, B., Ramel, C., and Dudler, R. (2010). Pseudomonas syringae virulence factor syringolin A counteracts stomatal immunity by proteasome inhibition. Mol. Plant Microbe Interact. 23:1287–1293.

Schuebel, F., Rocker, A., Edelmann, D., Schessner, J., Brieke, C., and Meinhart, A. (2016). 3’-NADP and 3’-NADP2, two metabolites formed by the bacterial type III effector AvrRxO1. J. Biol. Chem. 291:22888–22880.

Schwartz, S.H., León-Kloosterziel, K.M., Koornneef, M., and Zevaart, J.A. (1997). Biochemical characterization of the aba2 and aba3 mutants in Arabidopsis thaliana. Plant Physiol. 114:161–166.

Seo, M., KoIwai, H., Akaba, S., Komano, T., Oriani, T., Kamiya, Y., and Koshiba, T. (2000a). Abscisic aldehyde oxidase in leaves of Arabidopsis thaliana. Plant J. 23:481–488.
AvrRxo1 inhibits vitamin B6 biosynthesis, promoting infection in rice

Seo, M., Peeters, A.J., Koiwai, H., Oritani, T., Marion-Poll, A., Zeevaart, J.A., Koornneef, M., Kamiya, Y., and Koshiba, T. (2008a). The Arabidopsis aldehyde oxidase 3 (AOA3) gene product catalyzes the final step in abscisic acid biosynthesis in leaves. Proc. Natl. Acad. Sci. USA 97:12908–12913.

Shidore, T., Broeckling, C.D., Kirkwood, J.S., Long, J.J., Miao, J., Zhao, B., Leach, J.E., and Triplett, L.R. (2017). The effector AvrRxo1 phosphorylates NAD in planta. PloS pathog. 13:e1006442.

Su, J., Zhang, M., Zhang, L., Sun, T., Liu, Y., Lukowitz, W., Xu, J., and Zhang, S. (2017). Regulation of stomatal immunity by interdependent functions of a pathogen-responsive MPK3/MPK6 cascade and abscisic acid. The Plant Cell 29:526–542.

Thor, K., Jiang, S., Michard, E., George, J., Scherzer, S., Huang, S., Dindas, J., Derbyshire, P., Leitão, N., DeFalco, T.A., et al. (2020). The calcium-permeable channel OSCA1.3 regulates plant stomatal immunity. Nature 585:569–573.

Titz, O., Tambasco-Studart, M., Warzych, E., Apel, K., Amrhein, N., Laloi, C., and Fitzpatrick, T.B. (2006). Pdx1 is essential for vitamin B6 biosynthesis, development and stress tolerance in Arabidopsis. Plant J. 48:933–946.

Triplett, L.R., Shidore, T., Long, J., Miao, J., Wu, S., Han, Q., Zhou, C., Ishihara, H., Li, J., Zhao, B., et al. (2016). AvrRxo1 is a bifunctional type III secreted effector and toxin-antitoxin system component with homologs in diverse environmental contexts. PLoS One 11:e0158856.

Wagner, S., Bernhardt, A., Leuendof, J.E., Drewke, C., Lytovchenko, A., Mujahed, N., Gurgui, C., Frommer, W.B., Leistner, E., Fennie, A.R., et al. (2006). Analysis of the Arabidopsis rsr4-1/pdx1-3 mutant reveals the critical function of the PDX1 protein family in metabolism, development, and VB6 biosynthesis. Plant Cell 18:1722–1735.

Walter, M., Chaban, C., Schütze, K., Batistio, O., Weckermann, K., Nake, C., Blazevic, D., Grefen, C., Schumacher, K., Oecking, C., et al. (2004). Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. Plant J. 40:428–438.

Wang, S., Li, S., Wang, J., Li, Q., Xin, X.F., Zhou, S., Wang, Y., Li, D., Xu, J., Luo, Z.Q., et al. (2021). A bacterial kinase phosphorylates OSK1 to suppress stomatal immunity in rice. Nat. Commun. 12:5479.