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Phosphate starvation enhances *Xanthomonas oryzae* pv. *oryzae* resistance in rice

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Abstract

Bacterial leaf blight (BLB) is a common disease that affects rice development and yield. The effects of major nutrients, especially nitrogen, on rice BLB susceptibility have been considered when devising rational fertilization strategies. However, the defense mechanism of rice against BLB under phosphate (Pi)-deficient conditions remains uncertain. Jasmonic acid (JA) is a phytohormone produced by rice plants to respond to abiotic and biotic stresses. Here, the involvement of the JA pathway in rice response to *Xanthomonas oryzae* pv. o*ryzae* (*Xoo*) under low Pi was investigated in two contrasting rice cultivars G299 and G22. Expressions of JA-related genes under low Pi and Pi-related genes under JA treatment were assessed. The resistant capacity of G299 and G22 against *Xoo* infection was also investigated. In the JA-sensitive and Pi-sensitive cv. G299, JA-related genes were highly expressed under low Pi, and low Pi-responsive genes were strongly upregulated under JA treatment. Neither JA nor Pi pathways were activated in the JA-tolerant and low Pi-tolerant cv. G22. Low Pi strongly enhanced rice resistance to *Xoo* in cv. G299. Our study demonstrated that Pi deficiency confers rice resistance to *Xoo*. The JA pathway modulates the response to low Pi, depending on the cultivar. Pi-response genes are involved in Pi stress and may participate in the regulation of overall plant growth under various abiotic stresses. These findings provide new insights into the interaction between phosphate deficiency and the JA pathway and the subsequent effect on plant disease resistance.

Keywords: bacterial leaf blight, jasmonic acid pathway, phosphate starvation, rice, *Xanthomonas oryzae* pv. *oryzae*.

Introduction

Rice (*Oryza sativa* L.) is a staple food for more than half of the human population worldwide. The global population is expected to exceed nine billion by 2050; thus food production will need to be doubled (Hunter *et al.* 2017). Owing to climate change, the average global temperature is estimated to increase by 2 to 5°C in the next ten years (El-Sayed and Kamel 2020). These temperatures will allow favorable conditions for pathogen development, consequently affecting rice production. History has shown that mass crop failures can occur when climatic conditions are favorable for disease development (Burdon and Zhan 2020). In rice, bacterial leaf blight (BLB) is a common disease caused by *Xanthomonas oryzae* pv. o*ryzae* (*Xoo*); it seriously affects plant development and has the potential to severely reduce rice yield by up to 50% (Liu *et al.* 2014).

Inorganic phosphorus (Pi), is the second essential macronutrient, following nitrogen, for the growth and development of rice. However, the exclusive utilization of

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Abbreviations: AOC - allene oxide cyclase; AOS - allene oxide synthase; BLB - bacterial leaf blight; CML15 - calmodulin-related calcium sensor 15; COII - coronatine insensitive 1; Ile - isoleucine; J0 - medium without jasmonic acid; J1 - medium supplemented with 5 μM jasmonic acid; JA - jasmonic acid; JAR1 - jasmonate resistant 1; JAZ - jasmonate ZIM domain; LL - length of lesions; LOX - lipoxygenase; P0 - full Pi medium; P* - Pi starvation medium; PAP - purple acid phosphatase; PHR2 - phosphate starvation response 2; Pi - inorganic phosphorus; SPX - *SYG/PHO81/XPR1*; *Xoo* - *Xanthomonas oryzae* pv. *oryzae*.
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Pi fertilizers rapidly diminishes the nonrenewable Pi rock (Long *et al.* 2019). Therefore, several studies have focused on how to reduce phosphorus supply while maintaining crop yield. In rice, a number of low Pi-related genes have been described, including *phosphate starvation response 2* (*PHR2*) (Bustos *et al.* 2010), the negative regulator of *PHR2*, namely *SYG/PHO81/XPR1* (*SPX*) (Wang *et al.* 2009, Lv *et al.* 2014), *purple acid phosphatase* (*PAP*) (Zhang *et al.* 2011), and a *calmodulin-related calcium sensor 15* (*CML15*) which has been identified to be associated with the low Pi medium in our previous study (To *et al.* 2020). Moreover, in this study, the diverse response to low Pi conditions was also observed in 157 Vietnamese rice cultivars from different zones, ecosystems, maturity classes, *etc.* (To *et al.* 2019).

However, the association of Pi fertilizer with BLB has only recently been observed. Dong *et al.* (2019) suggested that OsPT8, a Pi transporter, was involved in rice disease resistance and PAMP-triggered immunity. In another study, overexpression of *OsPR10a*, encoding a plant pathogenesis-related protein purified from low Pi rice suspension-cultured cells, significantly enhanced the resistance to *Xoo* under Pi starvation conditions (Huang *et al.* 2016).

Under Pi-deficient conditions, plants have to find different strategies for Pi uptake from the soil and maintain P homeostasis *via* root system architecture. Different phytohormones were involved in the response of root architecture to low Pi supply, including auxin (López-Bucio *et al.* 2002, Pérez-Torres *et al.* 2008), ethylene (Zhang *et al.* 2003, Chacón-López *et al.* 2011), and cytokinin (Lai *et al.* 2007). The involvement of JA and its derivatives in response to Pi deprivation and the underlying molecular mechanism of this relationship has rarely been investigated in rice. JA is considered a phytohormone that responds to biotic and abiotic stresses, such as salinity (Zhao *et al.* 2014), heat, irradiance (Balfagón *et al.* 2019), heavy metals (Poonam *et al.* 2013), and pathogens (Campos *et al.* 2014, Zhang *et al.* 2017, Gupta *et al.* 2020).

Khan *et al.* (2016) found a relationship between the increased synthesis of JA and JA-Ile and Pi starvation in the culture medium of *Arabidopsis*. Similar associations were found in sorghum (Zhang *et al.* 2019) and cotton (Luo *et al.* 2021). Specifically, four genes involved in the JA response pathway, including one *COI1* gene and three *JAZ* genes, were strongly induced in the low Pi-sensitive sorghum cultivar. In contrast, these genes were weakly expressed in the low Pi-tolerant sorghum cultivar when grown in a Pi-deficient environment (Zhang *et al.* 2019). In cotton, five genes involved in the JA pathway, including *allene oxide synthase* (*GhAOS1/2*), *OPDA reductase* (*GhOPR3*), *ketoacyl-CoA thiolase* (*GhKAT*), *acyl-CoA oxidase* (*GhACX*), and *GhJAZ6/12* were strongly expressed when cotton plants were grown under low Pi supply; the JA and JA-Ile content also increased (Luo *et al.* 2021). Moreover, the activation of the JA signaling pathway may also help plants gain tolerance to *Spodoptera littoralis* in *Arabidopsis*, *Solanum lycopersicum*, and *Nicotiana benthamiana* (Khan *et al.* 2016), and *Verticillium dahliae* in cotton (Luo *et al.* 2021). Recently, the crosstalk between low Pi, JA signaling pathway, and resistance to *Xoo* infection was first reported in *O. sativa* L. var. *japonica* cv. Nipponbare (Kong *et al.* 2021). They demonstrated that low Pi promoted *OsMYC2* signaling - mediated JA pathway - to enhance rice defense against BLB *via* transcriptional regulation of *OsPHR2* on *OsMYC2*. These studies demonstrated that Pi starvation enhanced tolerance to some pathogens by activating JA biosynthesis in some plant species, providing new insights into the way how phosphate deficiency affects plant disease tolerance. However, the effect of different genetic backgrounds of rice plants on the response to *Xoo* under Pi deficiency condition remains uncertain.

In our study, first, we screened 20 rice accessions, which had contrasting responses to JA under low Pi conditions; then, we selected the two most contrasting rice cultivars regarding JA sensitivity and Pi starvation sensitivity. After that, the relationships among Pi starvation, JA pathway, and pathogen resistance were determined. Additionally, we evaluated the resistance of these rice cultivars to the *Xoo*. We expected that the new findings could contribute to a rational fertilization strategy in which rice plants could develop in conditions of reduced fertilizer and enhanced *Xoo* resistance thus maintaining yield under stress conditions.

Materials and methods

Plants and growth conditions: A population of 20 selected rice (*Oryza sativa* L.) cultivars with contrasting responses to JA was used in this study (Table 1 Suppl.). Their seeds were supplied by the Plant Resources Center in Hanoi, Vietnam.

To break down seed dormancy, seeds were first incubated for 5 d in an oven at 45°C. Thereafter, they were manually decorticated and sterilized with 70% ethanol for 2 min. Following rinsing several times with sterilized distilled water, seeds were immersed in 4% sodium hypochlorite. A few drops of *Tween 20* were added to the solution, and the solution was shaken every 5 min until 25 min. After that, seeds were washed six to seven times with sterilized distilled water and shaken to remove all hulls and bran. Seeds were subsequently left in water for 24 h and in the dark at 26°C to maximize water absorbance. Sterilized seeds were planted on Petri dishes of Murashige and Skoog (MS; *Duchefa*, Haarlem, Netherlands) MS/4 agar at 0.6% (m/v). After that, seeds were germinated at 37°C for 5 d.

Plantlets were grown in a culture room with a temperature of 28 - 30°C, a humidity of 70 - 80%, a 12-h photoperiod, and an irradiance of 350 µmol m^{-2} s^{-1} in a hydroponic tray containing two different Yoshida nutrient solutions: one contained a full Pi medium (P0) with 320 µM P, whereas the other contained a Pi starvation medium (P*) of 10 µM P (To *et al.* 2020). The culture medium was changed every week for two and six-week experiments. For the JA treatment, 2-d-germinated seeds were grown in MS/4 medium in a test tube supplemented with 5 µM JA (*Sigma-Aldrich*, St. Louis, USA) and

0 µM JA for the control (To *et al*. 2019). Plants were harvested after two weeks. The experiment was performed in triplicate. Each time, at least ten plantlets per treatment were used.

Plants were collected after six weeks of growing in a Pi starvation medium. To achieve a dry mass, plant materials were dried in an oven at 70°C for one week (To *et al*. 2020, Mai *et al*. 2021). Six different parameters were selected for the study: shoot dry mass, root dry mass, shoot length (the length of the longest leaf), root length (the length of the longest root), total dry mass, and the number of crown roots.

RNA isolation, cDNA synthesis, and real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis: The shoot and root samples of cvs. G22 and G299 were grown either in the test tube under control (0 µM JA) or 5 µM JA in the hydroponic culture with full and low Pi; samples were collected after two weeks (JA treatments) and two and six weeks (Pi treatments). Total mRNA was extracted using *TRIzol* reagent (*Thermo Scientific*, Waltham, MA, USA). The extracted mRNA was digested using *DNAse I* (*Thermo Scientific*) to remove DNA before cDNA conversion using the *MaximaR* first strand cDNA synthesis kit (*Thermo Scientific*). Quantitative PCR reactions were performed using the *qTOWER³* real-time PCR thermal cyclers (*Analytik Jena*, Jena, Germany). This experiment was performed in triplicate per sample per time point per treatment using the *GoTaq* RT-qPCR master mix (*Promega*, Madison, WI, USA). The qPCR was performed for two sets of genes related to the JA and Pi pathways. The primers used for qPCR were designed using the website https://www.ncbi.nlm.nih.gov/tools/primer-blast and were listed in Table 2 Suppl. Relative gene expressions were normalized to the reference gene (*actin*). The 15 µL reaction mix was composed of 4 µL 2× master mix (*Promega*), 250 nM forward primer, 250 nM reverse primer, 400 ng DNA template, and H_2O to make up the volume of 15 µL. The thermal cycling conditions were as follows: 95°C for 5 min, 40 cycles at 95°C for 30 s, 56°C to 58°C for 1 min, and 72°C for 1 min. The relative expressions of investigated genes were normalized against the expression of the housekeeping gene *OsActin* and analyzed by using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

Infection of rice plants with *Xoo*: Rice cvs. G22 and G299 were grown in sand supplemented with liquid Yoshida medium (To *et al*. 2020). The leaves of 6-week-old plants were artificially inoculated with *Xoo* using the clipping method (Kauffman *et al*. 1973). At least 10 plants were used in each treatment. Two days before inoculating rice plants, *Xoo* was sub-cultured by transferring the *Xoo* from the original solid PSA media (10 g L^{-1} peptone, 1 g L^{-1} glutamic acid, 10 g L^{-1} sucrose, and 20 g L^{-1} agar) to a new solid PSA media and incubating at 28°C for two days. On the day of inoculation, *Xoo* was suspended in the sterilized solution with 0.85% NaCl (pH = 7.0) to reach 1×10^{-8} cfu mL^{-1} (Ke *et al*. 2017). In each plant, three newly expanded leaves were clipped with sterile scissors dipped in this *Xoo* bacterial suspension. Leaves clipped with sterile scissors dipped in sterile distilled water served as the negative control. Following inoculation, leaves were covered with polyethylene bags to reach high humidity for 24 h. Plants were maintained at 27°C and 70% relative humidity during the study. The length of lesions (LL) was measured 14 d after inoculation. A second infection was performed on newly expanded leaves of the same plants 14 d after the first infection; LL was again measured 14 d after the second inoculation. LLs that were 0 - 5 cm, 5 - 10 cm, and >10 cm were considered tolerant, moderately tolerant, and sensitive to pathogens, respectively (Vikal *et al*. 2007). After 14 d and 28 d of inoculation, the quantity of bacteria in the leaves was determined using the method of Ke *et al*. (2017). Briefly, a 6 cm-infected leaf surface without the blight part was sterilized with 70% ethanol for 1 min before being ground into powder and homogenized in 1 mL *Milli-Q* water. The homogenate was diluted 10^5 to 10^6 times until appropriate dilution. The homogenate was spread in a solid peptone sucrose agar medium and incubated for 3 d at 28°C until obvious colonies formed. The experiment was performed in triplicate.

Statistical analysis: The differences between investigated parameters were statistically analyzed using the Student's *t*-test in *R program* software *version 3.6*.

Results

Twenty of the 157 rice cultivars previously found to respond contrastingly to jasmonic acid (To *et al*. 2019) were selected for this study (Fig. 1 Suppl.). Six morphological traits, including shoot length, root length, number of crown roots, shoot dry mass, root dry mass, and total dry mass, were selected to evaluate the effect of low Pi on the phenotypic appearance of the selected rice cultivars (Fig. 1). In general, there was a diverse response of rice plants grown under full and low Pi conditions for six weeks. Root length and root mass were positively affected by low Pi, while the four remaining traits, shoot length, number of crown roots, shoot mass, and total mass, were reduced under Pi starvation. The cv. G22 was among the top three cultivars that were least affected by Pi starvation among the six investigated traits. Thus, G22 was considered the cultivar most tolerant to Pi starvation. In contrast, G299 belonged to the top five cultivars strongly affected by Pi starvation for all six tested traits. Furthermore, in our previous study, these two cultivars differed contrastingly concerning Pi uptake and Pi use efficiency (To *et al*. 2020). Thus, G22 and G299 were considered the two most contrasting rice cultivars with respect to Pi starvation among the 20 selected cultivars and were consequently used to study gene expression.

Four representative genes involved in JA synthesis and signaling pathways, namely *allene oxide cyclase* (*AOC*),

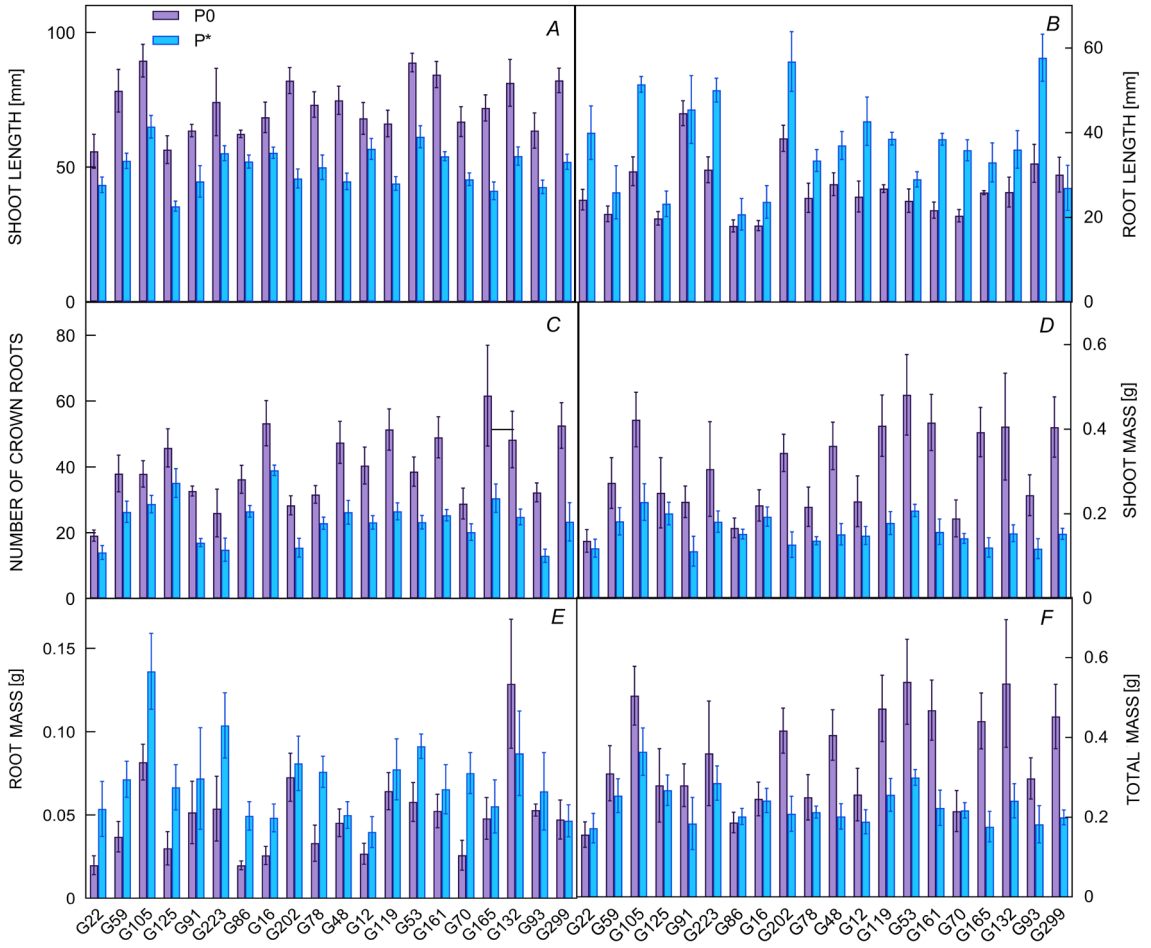

Fig. 1. Phenotypic response of 20 Vietnamese rice cultivars to 6-week-growth under full Pi (P0 - 320 µM P) and low Pi (P* - Pi starvation at 10 µM P) conditions presented by values of shoot length, root length, number of crown roots, shoot mass, root mass, and total mass. The order of the cultivars from left to right corresponds to the strong to weak resistance to jasmonic acid. Means ± SDs from three independent biological replicates.

jasmonate resistant 1 (*JAR1*), *jasmonate ZIM domain* (*JAZ5* and *JAZ8*), were selected, and their expression under Pi starvation for two weeks and six weeks were studied. The relative expression, which showed the results of how the expression of genes induced at low Pi condition related to the ones induced at high Pi condition, is shown in Fig. 2. All four tested genes were strongly induced under Pi starvation in the sensitive cv. G299 until the sixth week. The expression of *AOC* was approximately five times higher in the leaves of the cv. G299 in the Pi starvation medium than in the full Pi medium in 6-week-old plants. In this study, *JAR1* was induced approximately eight times in the leaves of 6-week-old G299 plants under low Pi than those under full Pi.

Moreover, the *JAZ* genes that encode the JA signaling receptors were also induced under low Pi conditions. Specifically, the *JAZ5* and *JAZ8* genes were induced approximately three and 37 times, respectively, in G299 rice plants grown under Pi starvation compared with those under full Pi after six weeks. Relative expression, which was less than two, was observed for these four investigated genes in the low Pi-tolerant G22 plants at either time point, indicating no significant induction. In 2-week-old plants, the expressions of *JAZ5* and *JAR1* were not significantly induced under low Pi. However, *JAZ8* and *AOC* were more than two times expressed in the leaves and roots of G299 plants grown for two weeks under Pi deprivation medium compared with those under full Pi treatment, respectively. Meanwhile, a marginal decrease in the expression was observed in G22 for all four investigated genes ($P < 0.05$).

To confirm the relationship between Pi starvation and the JA signaling pathway, plants were further grown in a 5 µM JA medium for two weeks. The expressions of four investigated genes involved in the Pi regulation pathway, *SPX1*, *CML15*, *PHR2*, and *PAP21b*, were studied. Notably, the expression of these genes was markedly upregulated in the sensitive cv. G299 under higher JA concentration (Fig. 3). Specifically, the expression of *SPX1*, *PHR2*, and *PAP21b* was strongly induced in G299 leaf tissue, while *CML15* was induced in G299 root tissue. Notably, *SPX1* and *CML15* expressions were nearly 15- and 10-fold higher, respectively, under 5 µM JA compared

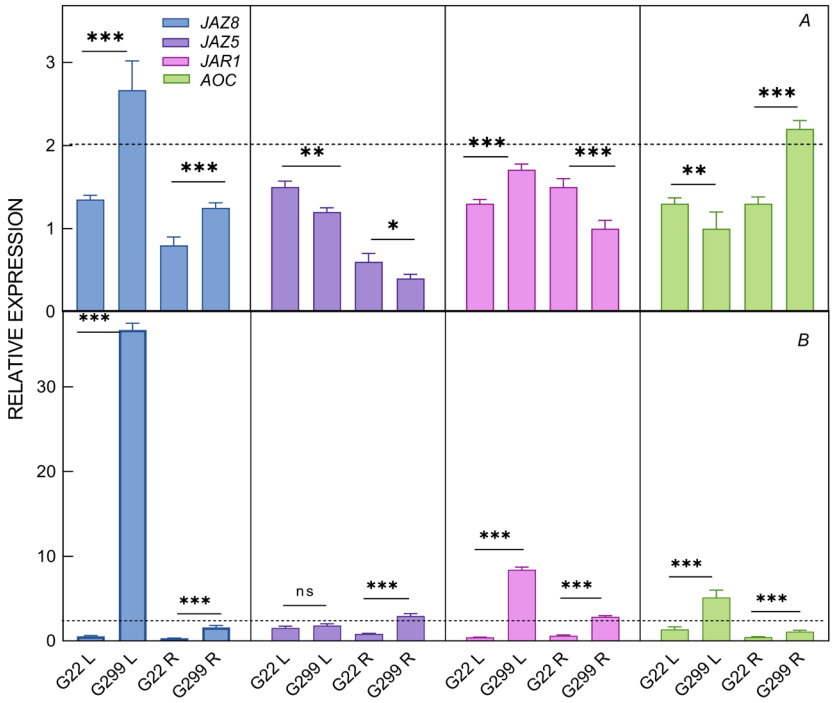

Fig. 2. Relative expression of JA-related genes of 2-week-old (*A*) and 6-week-old (*B*) plants grown under Pi starvation (10 µM P) compared with ones grown under full Pi (320 µM) in hydroponic culture medium. The y-axis indicates the relative expressions of four investigated genes. *The dotted line* indicates the value of 2. G22 (low Pi-tolerant rice accession), G299 (low Pi-sensitive rice accession). R - roots, L - leaves. Means ± SDs from three independent biological replicates. *, **, *** indicate a significant difference at $P < 0.05$, < 0.01, and < 0.001, respectively, ns - no significant difference according to Student's *t*-test.

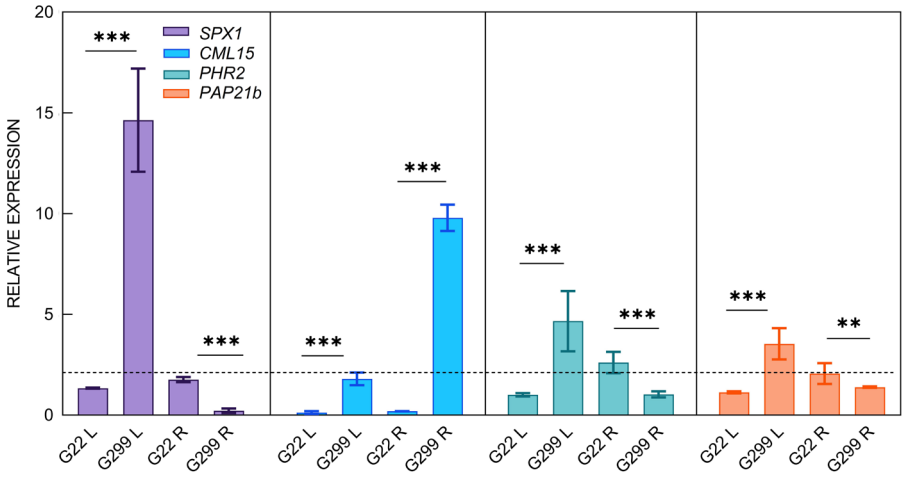

Fig. 3. Relative gene expression of Pi-related genes of 14-d-old plants grown under 5 µM JA compared with those grown in the medium without JA in the test tube. The y-axis indicates the relative expression of four investigated genes. *The dotted line* indicates the value of 2. G22 - low Pi-tolerant rice accession, G299 - low Pi-sensitive rice accession, R - roots, L - leaves. Means ± SDs from three independent biological replicates. *, **, *** indicate significant difference at $P < 0.05$, < 0.01, and < 0.001, respectively, according to Student's *t*-test.

to the control. An extremely low induction of the four Pi-related genes was observed in the low Pi-tolerant and JA-tolerant G22 under 5 µM JA.

The relationship between Pi starvation and the JA pathway was investigated by infecting the two contrasting rice cultivars with *Xoo.* Two weeks post-infection, the 8-week-old plants were tested. The second infection was performed on the same plants with newly developed leaves two weeks after the first infection. The results were also determined two weeks after the second infection. Images of leaves after the two infections are presented in Fig. 4. The results showed that, in the cv. G299, shorter lesion length (LL) was observed when plants were grown under Pi deprivation than under full Pi ($P < 0.001$). The LL

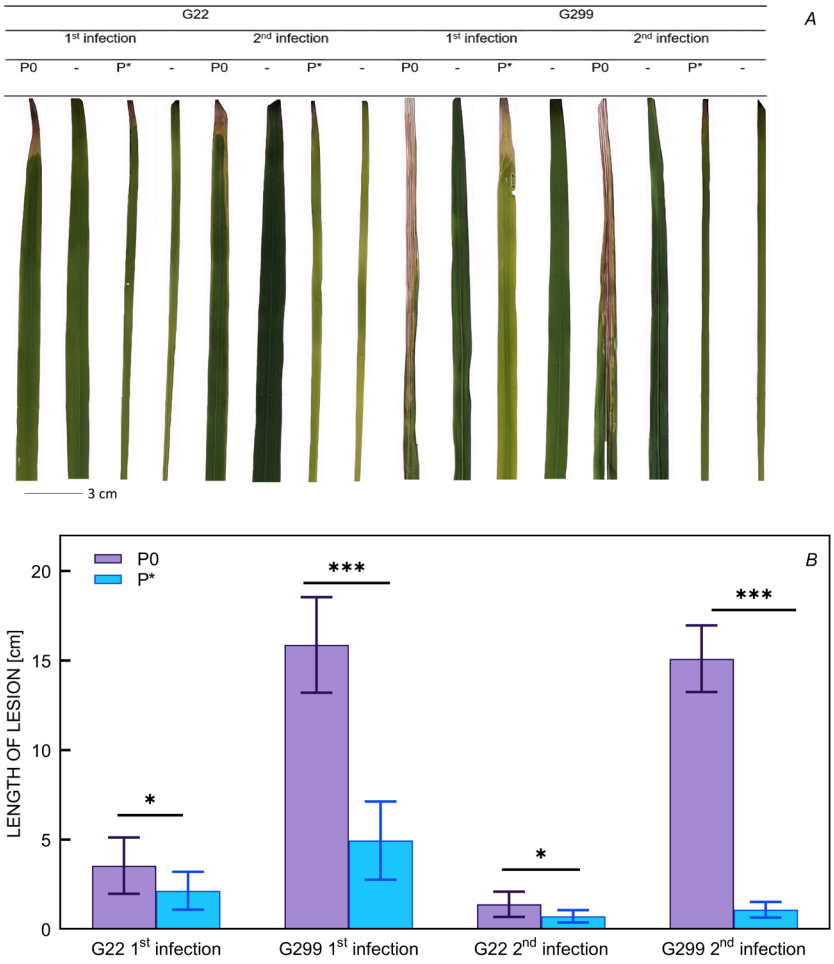

Fig. 4. *A* - Leaves of low Pi-tolerant cv. G22 and low Pi-sensitive cv. G299 at 1st infection and 2nd infection by *Xanthomonas oryzae* pv. *oryzae*. Plants were grown under sufficient Pi supply (P0) or under Pi starvation (P*). *B* - Length of lesion on leaves at 1st infection and 2nd infection. Means ± SDs from three independent biological replicates. * and *** indicate significant difference at $P < 0.05$ and < 0.001, respectively, according to Student's *t*-test.

was shorter after the second infection than after the first infection, suggesting that rice plants may further activate their defense mechanisms to reduce disease symptoms. In the first infection, under low Pi, the cv. G299 became moderately resistant with LL belonging to the range from 5 to 10 cm. In the second infection, under low Pi conditions, G299 became resistant to *Xoo* with LL < 5 cm. In P0 medium, G299 was sensitive to *Xoo*, with an LL of approximately 15 cm in both the first and second infections. Regarding the low Pi-tolerant and JA-tolerant cv. G22, this cultivar was resistant to *Xoo* under control conditions (LL < 5 cm). Moreover, the LL was shorter under low Pi than under control conditions. Furthermore, G22 became more resistant to *Xoo* during the second infection.

The *Xoo* quantification also confirmed the results of lesion length measurement. The highest quantity of *Xoo* per 1 cm leaf (approximately 1.32×10^9 cfu) was obtained in cv. G299 in the first infection when they were grown in P0 medium. In both cvs. G22 and G299, the *Xoo* amount was lower when the plants were grown in the P* medium than in the P0 medium in both the first and second infection. The lowest *Xoo* quantity was found in G22 plants grown in P* medium in the second infection (Fig. 5).

Discussion

This study systematically investigated the response of rice to the pathogen *Xoo* by studying the expression of JA-related genes and Pi-related genes under Pi starvation and JA treatment, respectively. Two Vietnamese rice cultivars with contrasting JA and Pi starvation responses were used for comparison. The results revealed the interplay between Pi deficiency, the JA pathway, and the pathogen tolerance in rice.

Twenty selected rice cultivars that responded contrastingly to JA were used to study their response to a low Pi medium. Results showed a diverse response regarding morphological traits to the low Pi condition, depending on the rice genetic background. The diverse response to low Pi has been observed in other studies where the cv. G22 belonged to the top three with the highest

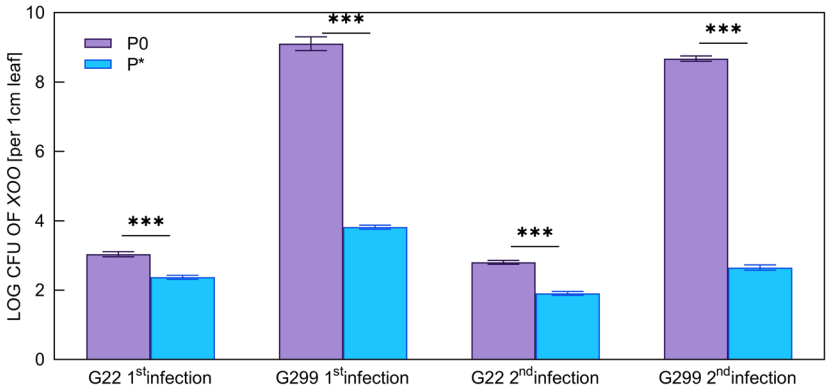

Fig. 5. *Xoo* quantity [log cfu cm^{-2}] in the leaves of the low Pi-tolerant G22 and the low Pi-sensitive G299 cultivars at 1st infection and 2nd infection by *Xanthomonas oryzae* pv. *oryzae*. P0 - full Pi at 320 µM P, P* - Pi starvation at 10 µM. Means ± SDs from three independent biological replicates. ** and *** indicate significant differences at $P < 0.01$ and < 0.001, respectively, according to Student's *t*-test.

phosphate use efficiency in both P0 and P* medium. In contrast, the cv. G299 cultivar was among the top three with the lowest phosphate use efficiency (To *et al.* 2020, Mai *et al.* 2021). However, regarding JA response, cvs. G22 and G299 responded similarly. These results suggest that these two rice cultivars responded differently to Pi stress.

These two most contrasting cultivars (G22 and G299) were selected to study how the genes involved in the JA and Pi pathways were regulated. In the Pi starvation medium, the expressions of four JA-related genes, *AOC*, the gene involved in the biosynthesis of JA in the chloroplast, *JAR1*, the gene that converts JA to JA-Ile in the cytosol, and two *JAZ* genes (*JAZ5* and *JAZ8*), the genes that regulate the JA-signal response, were investigated. All investigated genes were strongly induced under low Pi in the JA-sensitive and low Pi-sensitive cv. G299, while no significant change was observed in the JA-tolerant and low Pi-tolerant cv. G22. Similar results were observed for sorghum, where *JAZ10*, *JAZ11*, *JAZ18*, and *COI1* were strongly induced in the low Pi-sensitive sorghum cultivar. In contrast, no significant induction of these genes was observed in the low Pi-tolerant cultivar (Zhang *et al.* 2019). Therefore, the JA pathway is involved in the response of low Pi-sensitive plants. Similar results were observed for *Arabidopsis thaliana*, cotton, and rice. In a study using the RNA-seq results of *Arabidopsis thaliana*, Chevalier *et al.* (2019) observed an upregulation of JA biosynthesis and signaling-related genes under low Pi conditions. Another study showed that *AtJAZ10* expression increased nearly 4-, 5-, and 17-fold in shoots, roots, and root tips, respectively, under Pi starvation conditions (Khan *et al.* 2016). Two marker genes of JA biosynthesis, *VSP2*, and *LOX2*, were also induced in root tips and roots of Pi-deficient plants. JA and JA-Ile content increased in plants grown in a Pi-deficient medium (Khan *et al.* 2016). In cotton, the expressions of *GhAOS1/2*, *GhOPR3*, *GhKAT*, *GhACX*, and *GhJAZ6/12* were increased under low Pi in both the RNA-seq analysis and qPCR results. Moreover, JA and JA-Ile content increased 1.4- and 2.1-fold, respectively, compared to that under control conditions (Luo *et al.* 2021). Only one study reported the involvement of *OsJAZ11* in response to low Pi stress in a Pi-sensitive rice cultivar (PB1) (Pandey *et al.* 2021). These findings are consistent with our results for sensitive plants.

However, in rice, only one study reported the link between Pi and JA, where it showed that *OsPHR2* promoted *OsMYC2* signaling. Our study showed that after both two weeks and six weeks of growth under low Pi conditions, the genes involved in the JA pathway were not induced in the Pi starvation tolerant cv. G22. These results suggest that JA is only one of the ways in which rice plants deal with low Pi stress; however, the activation depends on the genetic background of the plant. Another hormonal pathway could be involved in plant immunity in the JA-insensitive cultivar. For example, auxin- and ethylene-responsive genes are activated in tolerant plants yet not in sensitive plants (Jiang *et al.* 2017). The β-expansin gene, which encodes a cell wall protein, could also contribute to low Pi tolerance in *Arabidopsis* and maize. The low Pi-tolerant maize also activates high expression of the β-expansin gene; however, this situation was not observed in sensitive maize (Guo *et al.* 2011, López-Arredondo *et al.* 2014, Jiang *et al.* 2017). Thus, we suggest that JA plays a modulatory role in regulating the response of plants under conditions of stress.

Furthermore, to investigate the association between JA- and Pi-responsive pathways, two rice cultivars with contrasting responses to low Pi were grown in the presence of JA, and the expression of four Pi-responsive genes was quantified. Notably, we observed an upregulation of *SPX1*, *CML15*, *PHR2*, and *PAP21b* in a JA-sensitive cultivar, whereas no change or little change in their expression was noted in the JA-tolerant cultivar. *PHR2* enhances the expression of phosphate starvation-induced (PSI) genes, resulting in enhanced Pi acquisition under low Pi (Lv *et al.* 2014). In rice, *SPX1*, *SPX2* (Wang *et al.* 2009), *SPX4* (Lv *et al.* 2014), and *SPX6* (Zhong *et al.* 2018) were specifically induced by Pi deprivation. In our study, JA treatment led to high expression of *SPX1* and *PHR2* genes in 14-d-old plants showing the relationship between these two abiotic stresses. Furthermore, a common strategy to deal with low Pi is the secretion of purple acid phosphatases

(PAP). In rice, 26 *PAP* genes were identified, of which many were significantly induced under Pi starvation (Zhang *et al.* 2011). Recently, Mehra *et al.* (2017) also showed that *OsPAP21b* primarily responds to Pi deficiency, is upregulated in this medium, and is transcriptionally controlled by *OsPHR2*. Notably, in rice plants stressed by JA, *PAP21b* also increased by approximately four-fold in the low Pi-sensitive cultivar to that in the control medium without JA, demonstrating that *PAP21b* was also involved in response to JA stress. *CML15*, which is involved in calcium signaling transduction, increased expression by approximately 10-fold in plants treated with JA compared to the negative control, also showing the participation of this gene in coping with JA stress. Overall, our study shows that the four low Pi-responsive genes were activated in the JA-sensitive cultivar, showing the involvement of the low Pi-responsive genes in response to JA stress, participating in the regulation of overall plant growth under abiotic stress. This is the first study to show the involvement of a low Pi-responsive pathway in response to exogenous JA in rice and opens up an avenue for future research. For the following steps, more studies should be implemented to deeply investigate how the low Pi-responsive genes can be involved in JA responsive pathway.

Two rice cultivars were grown under Pi starvation and infected with a *Xoo*. Disease symptoms were measured 14 d after the first and second infection. The LL was significantly ($P < 0.05$) reduced in plants grown under low Pi compared to that of plants grown under full Pi during the first infection, indicating that under Pi starvation plants also activated a mechanism to help plants to respond to pathogens. The *Xoo* quantification also confirmed the results obtained from the LL measurement. The amount of *Xoo* was lower in both rice cultivars when they were grown in the Pi deficiency medium than in the full Pi medium. The increased resistance of the Pi-starving plants to *Xoo* is ascribed to the accumulation of JA where we observed a strong induction of the JA-signaling-pathway genes in low Pi-sensitive cv. G299. These results were similar to those reported in *Arabidopsis thaliana*, *Solanum lycopersicum*, *Nicotiana benthamiana* (Khan *et al.* 2016), and cotton (Luo *et al.* 2021), where the JA pathway was activated in response to *Spodoptera littoralis* and *Verticillium dahliae*, resulting in a decrease in disease symptoms, and the caterpillar mass. In both JA-sensitive and JA-tolerant cultivars, LL as well as the *Xoo* quantity in the leaves were also reduced at the second infection with *Xoo*. This was because induced resistance mechanisms such as the JA pathway and other defense mechanisms were likely to be activated, which led to an increase in the defense response and decreased LL. This finding confirms the association between Pi deficiency and enhanced pathogen resistance *via* the JA pathway in low Pi-sensitive rice cultivars. However, low Pi-tolerant cultivars, which have different genetic backgrounds compared to low Pi-sensitive cultivars, may have other mechanisms to cope with *Xoo* in a constitutive or inducible manner (Freeman and Beattie 2008). The same stress performed in different plant cultivars may activate or inactivate different genes owing to differences in genetic background, leading to diverse responses (Oono *et al.* 2013, Jiang *et al.* 2017). Further studies should be performed afterward to investigate the mechanisms behind how the different genetic background affects the response of rice plants to pathogens differently. In the next steps, the JA-pathway knocked out should be performed, and the response of rice plants to *Xoo* should be studied again in order to check whether the JA pathway is really involved in the resistance of rice plants to *Xoo* or not.

Conclusions

First, our study showed tolerance to the pathogen *Xanthomonas oryzae* pv. *oryzae* in rice can be conferred under Pi starvation conditions. The activation of the JA pathway under low Pi led to the resistance of rice plants to the pathogen *Xoo*, which was dependent on the genetic background of the rice cultivar. Furthermore, the low Pi-responsive pathway was activated during JA stress. These results suggest that low Pi-responsive genes are involved in plant growth under this abiotic stress. This study provides insight into the interactions between nutrient supply and the defense systems of rice plants. These results could assist farmers in devising a rational fertilization strategy based on their genetic background.

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