Systemic adaptation of rice plants under low phosphate conditions and interaction with endophytic bacteria

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Highlights

- *The low phosphate-containingmedium significantly increased root length, root weight, and the number of crown roots of rice plants*
- *Rice plants grown in the P+113 medium produced higher levels of amino acids than in other conditions.*
- *The roots of the low-phosphate-sensitive-G299 rice plants grown in the P+113 medium showed the highest relative expression of phosphate uptake and metabolism genes.*
- *Expression of OsJAZ5 was significantly decreased in the roots of the low-Pi-sensitive G299 rice plants grown in the P+205 medium.*

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See online Appendix for additional Figures.

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Abstract

Phosphate (Pi) is essential for plants. Plants have adapted mechanisms to overcome Pi deficiencies. This study examined the interaction of two contrasting rice varieties (G22 and G299) and two endophytic bacterial strains. Four different culture media were established: full Pi (P0), Pi starvation (P*), insoluble Pi with Pi-solubilizing *Burkholderia* sp. strain 205 (P+205), or Pi-insolubilizing strain 113 (P+113). We investigated the responses of rice to these media. Root length and weight and the number of crown roots were higher in the P* and P+113 medium than the two other media. However, shoot length, and weight were lower. Most amino acid families were higher in the P+113 medium than in the other media. The roots of G299 plants in the P+113 medium showed the highest relative expression of all phosphate-analyzed genes; however, these genes were expressed at low levels in the leaves of both rice varieties. Notably, the jasmonic acid gene *OsJAZ5* showed the highest expression in the roots of G299 plants in the P+113 medium. Our results demonstrate the strong effects of the different genetic backgrounds of bacteria and rice plants on the response to low Pi. We also demonstrate the involvement of jasmonic acid in low Pi and soluble-phosphate-bacteria interaction in G299 plants. A positive interaction between *Burkholderia* sp. strain 205 and rice plants has been noticed in the promotion of plant growth. Further studies under field conditions should be undertaken to develop this potential strain as a biofertilizer.

Introduction

Rice plants (*Oryza sativa L.*) require phosphate (Pi) for their normal growth and development. Structurally, Pi is a major component of many important structures, such as DNA, RNA, ATP, and cell membranes. Functionally, Pi is involved in many metabolic pathways, including energy transfer, protein activation, and carbon and amino acid metabolic processes (Wu *et al*., 2003). However, it is relatively inaccessible to plants because of its low solubility and relative immobility in agricultural soils (Hirsch *et al.*, 2006). Pi is usually fixed with aluminum, iron (Lindsay, 1979), and calcium (Haefele *et al.*, 2014), resulting in approximately 70% of global croplands being Pi deficient (Kirkby and Johnston, 2008). Almost 75% of the applied phosphorus fertilizer forms complexes with soil and becomes unavailable for plants

(Ezawa *et al.*, 2002). Moreover, the overuse of phosphate fertilizers to solve the low Pi problem has led to water and soil pollution, resulting in algal blooms in the water environment (Alori *et al.*, 2017). Therefore, crops are frequently subjected to Pi deficiency, which can adversely affect many cell metabolic processes.

Plants have developed multiple mechanisms to cope with low Pi levels. Some crops show multiplication of lateral roots and root hairs (Jiang *et al.*, 2007), a decrease in shoot growth, secretion and release of organic acids (Mengel *et al*. 2001), development of mycorrhizal symbiosis (Kariman *et al.*, 2014; Tawaraya, 2022), and activation of low-Pi-responsive genes (Paz-Ares *et al.*, 2022). Several important genes and proteins involved in the low Pi responsive pathway in rice have been identified and studied, including *Phosphate starvation response2* (*PHR2*) (Zhang *et al.*, 2011a), *Phosphate1* (*PHO1*) (Secco *et al.*, 2010), SYG1/Pho81/XPR1 (SPX) domain-containing proteins (Wang *et al.*, 2009), phosphate transporter PT9 (Wang *et al.*, 2014) and purple acid phosphatases (PAPs) (Zhang *et al.*, 2011b). Phosphate transporters (OsPTs) and the phosphorus starvation response (OsPHR) are thought to play a crucial role in Pi starvation (Kumar *et al.*, 2021). Jasmonic acid genes have also been shown to be involved in response to nutrient starvation, including macroelements (N, P, K) and microelements (Fe, Zn) in rice and chickpea (Singh *et al*., 2015; Kobayashi *et al*., 2016). Recent advances in analytical technologies have also revealed new insights amino acid involvement in coping with stress, showing the systemic adjustment of plants to stress conditions (Chea *et al.*, 2021; Bechtaoui *et al.*, 2021). Specifically, proline which was well-known in response to osmotic stress caused by drought or salinity, has also been demonstrated to be involved in the response to Pi starvation. The proline concentration started to increase after 7 d of Pi starvation, and it was seven times higher than in control after 14 d when wild-type *Arabidopsis* plants were cultured on Pi starvation medium. Moreover, the expression of genes that control proline metabolism were also considerably increased by Pi starvation.

When Pi is fixed with metal ions, there are some microorganisms, including *Bacillus* (Wang *et al.*, 2017), *Pseudomonas* (Babalola and Bernard, 2012; Mei *et al.*, 2021), *Rhizobium* (Tajini *et al.*, 2012), *Penicillium*, and *Aspergillus* (Wang *et al.*, 2017), which can increase the availability of Pi by solubilizing insoluble Pi to become soluble Pi available absorption by plants. Their mechanisms include acidification by producing organic acids (Mei *et al.*, 2021), ion exchange, chelation (Alori *et al.*, 2017), and acid phosphatase secretion (Barra *et al.*, 2018). The Pi-solubilizing microorganisms have been applied to diverse plant species, such as *Bacillus megaterium* bacteria in mulberry (Baqual and Das, 2006), *Pseudomonas* and *Bacillus* bacteria in wheat (Charana Walpola, 2012), *Rahnella* sp. and *Burkholderia* sp. in poplar (Varga *et al.*, 2020), *Pantoea agglomerans* IALR1325 in pepper, and tomato (Mei *et al.*, 2021). The results showed better plant growth under low Pi conditions. Interestingly, this has also been applied to rice (Chen and Liu 2019; Raj 2014; Stephen *et al*. 2015). Rice plants inoculated with Pi-solubilizing bacteria showed significantly increased plant height, biomass, root growth, and Pi uptake (Bargaz *et al*., 2021). Recently, using these microorganisms has become a promising strategy to improve soil fertility while protecting the environment, thus sustaining food production worldwide. Several studies have pointed out that the need for Pi fertilizers can be reduced by up to 50% without compromising yield using phosphate-solubilizing microorganisms in maize (Jilani *et al.*, 2007).

Therefore, this study aims to understand the different genetic backgrounds of rice in their response to different Pi conditions by supplementing media with soluble and insoluble-Pi bacteria. Two rice varieties, including the low-Pi-tolerant variety G22 and the low-Pi-sensitive variety G299 were used as the plant materials. Morphological and biochemical changes in these rice varieties under low Pi culture conditions and in the medium supplemented with endophytic *Burkholderia sp*. bacteria were analyzed. The colonization of bacteria inside the roots was recorded. Finally, the relative expression of genes involved in the low-Pi-adapted processes were quantified. The results from our study provide new insights into the interaction between rice plants and endophytic bacteria under various levels of Pi-deficient conditions and suggest strategies to select rice varieties with a tolerance of low Pi stress for sustainable use of plant genetic resources in agriculture.

Materials and Methods

Plant materials

Two selected rice varieties, including (Trung Trang Tuyen Quang belong to *Indica* group, traditional plant, VNPRC code is 760), and G299 (Blao sinh sai belongs to *Japonica* group, traditional plant, VNPRC code is 4806), were selected for this study. Their seeds were supplied by the Plant Resources Center in Hanoi, Vietnam.

Bacteria strains

Two bacterial strains identified as *Burkholderia sp*. strain 205 (bacteria can solublize Pi at a very high rate, P+205) and the control *Burkholderia sp*. strain 113 (bacteria cannot solubilize Pi, P+113) were selected from our previous study (unpublished data). The strains were isolated from the rhizosphere and endosphere of local rice plants, and their Pi-solubilizing capacity was tested i*n vitro* against $Ca_3(PO4)_2$. The *Burkholderia* bacteria stock, kept at –80ºC in liquid Luria-Bertani (LB) medium containing 25% of glycerol, was plated in solid LB medium for 3 days at 28°C. Then, a single colony of *Burkholderia* was grown in 10 ml of LB medium for a further 24 h at 28°C to produce the fresh bacteria solution. Overnight bacterial cells were then centrifuged for 5 min at 4000 rpm and resuspended in sterile distilled water to reach OD_{600} = 1. This bacteria solution was used to inoculate with the rice plants (King *et al*., 2019).

Plant growth conditions

To break down seed dormancy, seeds were first incubated for 5 d in an oven at 45°C. They were then manually decorticated and sterilized with 70% ethanol for 2 min. After rinsing several times with sterilized distilled water, the seeds were immersed in 4% sodium hypochlorite. A few drops of Tween 20 were added to the solution, which was shaken every 5 min for 25 min. The seeds were then 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 in the dark at 26°C to maximize water absorbance. Sterilized seeds were planted on Petri dishes containing a quarter of solid Murashige and Skoog medium (MS; Duchefa, Haarlem, Netherlands) supplemented with 0.6% w/v agar. The seeds were then germinated at 37°C for 5 d in test tubes (To *et al*., 2019).

After 5 d, individual plants were grown in a culture room at a temperature of 28-30°C, approximately 70-80% humidity, and in a hydroponic test tube containing two specific Yoshida nutrient conditions (Yoshida *et al.* 1971): one contained a full Pi medium (P0) of 320 µM P (0.05 g/L NaH_2PO_4.H2O), whereas the other con-

tained a Pi starvation medium (P*) of 10 µM P (1.56 mg/L $NaH_2PO_4.H2O$) (To *et al.*, 2020). The insoluble-Pi-containing medium (P+) was prepared by replacing $NH_4H_2PO_4$ with $Ca_3(PO_4)_2$ with the same Pi concentration as the full Pi medium. After 1 week, 1mL of suspension of one-day bacteria *Burkholderia sp*. strain 205 and the control *Burkholderia sp*. strain 113 with OD_{600}=1 was added to the liquid culture medium of the rice plants for co-cultivation. The culture medium was filled every week for 6 weeks. Plants were harvested after 6 weeks. The experiments were performed in triplicate. Plantlets per treatment consisted of at least ten plants at each time point.

Sample collection and phenotypic analysis

Plants were collected after 6 weeks of growth in P0, P*, and insoluble Pi supplemented with *Burkholderia sp*. strain 113 (P+113) or *Burkholderia sp*. strain 205 (P+205) medium. After harvesting, plant materials were dried in an oven at 70 °C for 1 week to achieve a constant weight (To *et al.*, 2020; Mai *et al.*, 2021). Five specific parameters were selected and measured manually for the study: shoot weight (SHW, the dry weight of the shoot), root weight (RTW, the dry weight of roots), shoot length (SHL, the length of the longest leaf), root length (RTL, the length of longest root), and the number of crown roots (NCR).

Determination of free amino acid content

Shoot samples (100 mg dry weight) were crushed in liquid nitrogen and extracted with 1.5 mL of boiling Mili Q water. First, the mixture was placed in a boiling water bath for ultrasonication for 15 min and then incubated at 100 °C for 45 min with gentle agitation on a shaker (Eppendorf, Germany). The sample was filtered through a 0.2 µm pore membrane filter (Sartorius AG, Germany) (Jia *et al.*, 2018). The amino acid profile was determined using an amino acid analyzer (Biochrom 30+, Biochrom, Cambridge, UK) based on ion-exchange chromatography. Colorimetric detection was accomplished at 570 and 440 nm (for proline and N-leucine) after post-column derivatization with the ninhydrin reagent. Standard amino acids were purchased from Thermo Scientific. The total free amino acid content was calculated as the sum of each free amino acid content.

Determination of phosphate content

The phosphate concentration in the samples was analyzed following the protocol described by To *et al*. (2020) using the vanado-molybdophosphoric acid colorimetric method. The Pi content in the shoot samples (mg) was calculated by multiplying the Pi concentration (mg/g) in each sample by the SHW (g).

Localization of bacteria in plant roots

The movement of *Burkholderia* bacteria from the culture environment to the plant roots was visualized using a LSM900 confocal laser scanning microscope (Carl Zeiss, Germany). The time series were set at day 0, 7 d, and 14 d. The *Burkholderia* bacteria were transferred with a pIN29 vector containing DsRed controlled by a constitutive TAC promoter via heat shock transformation. Root samples were transversely cut to a thickness of 10 µm and visualized directly under the ZEISS LSM 900 confocal microscopy equipped with a 63× objective. The roots and bacteria were illuminated at 488 nm (autofluorescence) and 561 nm (bacteria).

Analysis of gene expression using real-time quantitative reverse transcription-polymerase chain reaction

Leaf and root samples of the G22 and G299 varieties grown in the Yoshida hydroponic culture with full and low Pi were collected after 6 weeks. Total mRNA was extracted from samples using TRIzol reagent (Thermo Scientific, Waltham, MA, USA). RNA samples with an A260/A280 ratio of 1.8–2.2 and an A260/A230 ratio>1.8 were used for further analysis. We synthesized cDNA from mRNA using the MaximaR First-Strand cDNA Synthesis Kit (Thermo Scientific). Go Taq RT-qPCR Master Mix (Promega, Madison, WI, USA) was used for quantitive polymerase chain reaction (qPCR). The Q-Tower 3 Analytik Jena qPCR system (Germany) was used to quantify and compare the expression of the investigated genes in the selected rice variety grown in two distinct culture media. The experiment was performed in triplicate per sample, per time point, and per treatment. Primers were designed using the website https://www.ncbi.nlm.nih.gov/tools/primer-blast and then synthesized. Relative gene expression levels were normalized to the reference gene (actin). We added 4 µL 2× master mix (Promega), 250 nM gene-specific forward primer, 250 nM gene-specific reverse primer, 400 ng DNA template, and H_2O to make up the total volume to 15 µL. The thermal cycling qPCR conditions were as follows: 95°C for 5 min, 40 cycles of 95°C for 30 s, 56°C to 58°C for 1 min, depending on genes, and 72°C for 1 min. Product specificity was tested using a melting curve analysis (60-95°C). Relative gene expression under selected conditions was calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). The sequences of the primers used for qPCR are presented in Table S1.

Statistical analysis

All experiments were performed in triplicate. The data are presented as the mean ± standard deviation from three replicates. The differences between the investigated parameters were statistically analyzed using one-way ANOVA and the Tukey post-hoc test in the R program, software version 3.6.

Results

Morphological changes of rice plants under various phosphate conditions

Overall, the results showed that bacteria affected the growth of plants in both selected rice varieties. There was also a significant difference in the effects of the two bacterial strains on the development of the rice plants ($P<0.05$).

In terms of RTL, RTW, and NCR, we obtained significantly higher values in the plants grown in P* and P+113 than in P0 and P+205 ($P<0.05$) (Figure 1). Conversely, regarding SHL and SHW, lower values of these two parameters were obtained in the plants grown in the Pi-deficient-medium (P*) and P+113, showing that rice plants developed abnormally in the Pi-deficient medium, resulting in reduced growth.

A significant difference was observed in the effect of *Burkholderia sp*. strains 113 and 205 on the growth of rice plants. *Burkholderia sp*. strain 205 can convert insoluble Pi into soluble Pi; therefore, it can supply sufficient Pi to plants. Supplementing the *Burkholderia* sp. strain 205 into the -Pi-insolubilizing-containing medium increased the SHL and SHW and reduced RTL, RTW, and NCR compared to those in the medium supplemented with *Burkholderia sp*. strain 103.

A similar trend was also observed for the G22 variety. However, owing to the different genetic backgrounds of the plants, the development of the six investigated parameters in the G22 and G299 rice varieties differed. The RTL, RTW, and NCR

were significantly higher in the G22 variety than in the G299 variety (P<0.05). However, shoot length was significantly lower in the G22 variety than in the G299 variety (P<0.05). There was no significant difference in the SHW and TTW between the G22 and G299 rice varieties (P>0.05; Figure S1). Remarkably, SHW was reduced by 30% in the Pi-deficient-medium (P*) in the low-Pi-sensitive G299 variety, whereas SHW was reduced by only 16% in the low-Pi-tolerant G22 variety. RTL and RTW increased by approximately 54% and 72%, respectively, in G299, whereas they increased only 27% and 36%, respectively, in the G22 variety in P* medium.

Colonization of bacteria in the rice roots

The colonization of *Burkholderia* sp. bacteria in the G299 rice root system was investigated. The confocal images showed that the root was initially free of bacteria (Figure 2A). After inoculating roots

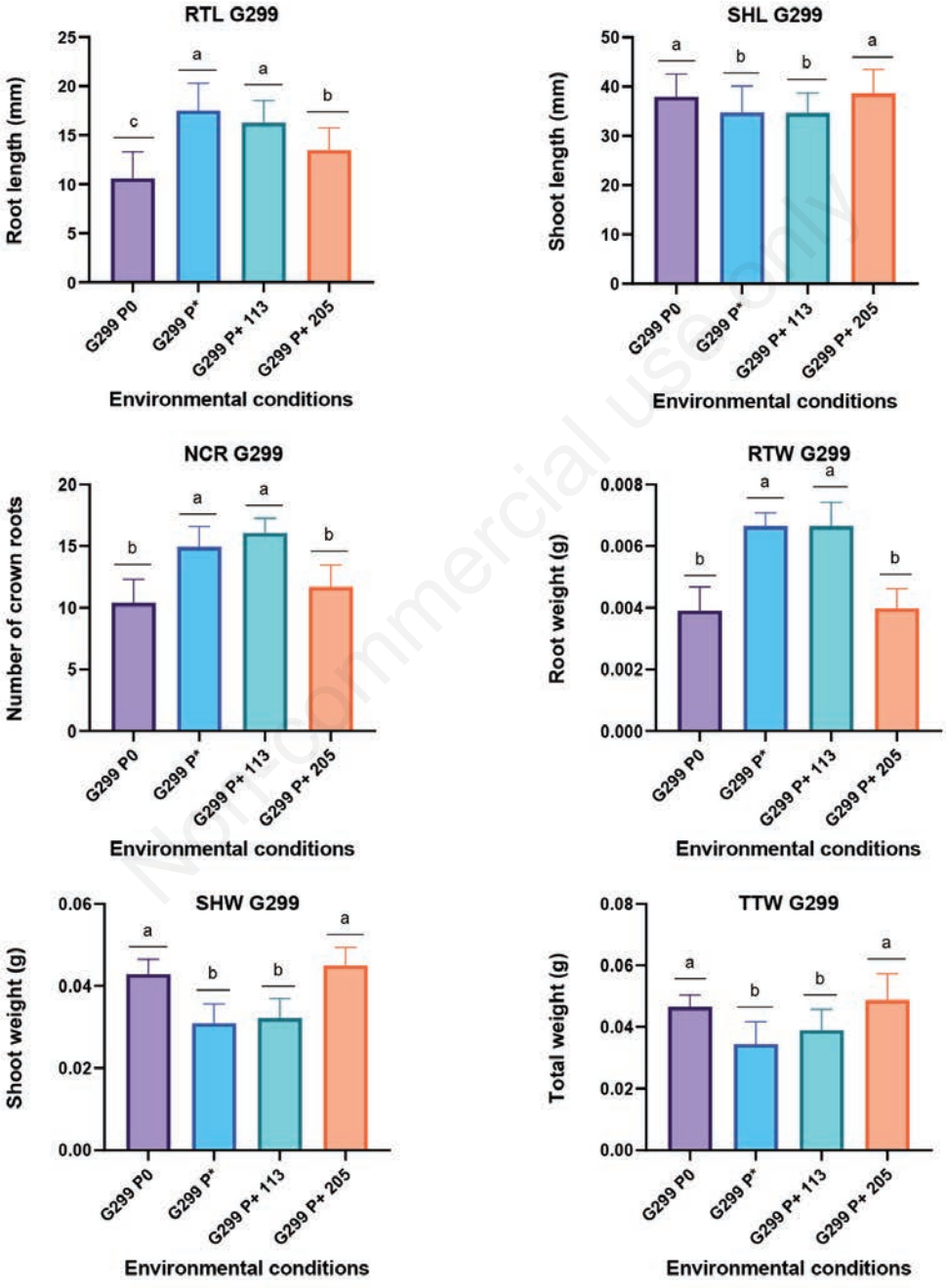


Figure 1. Morphological adaptation of G299 rice plants grown under different culture conditions. P0 (full Pi), P* (low Pi), P+113 (insoluble Pi supplemented with *Burkholderia* sp. strain 113), P+205 (insoluble Pi supplemented with Burkholderia sp. strain 205). Different letters indicate significant differences between treatments (P<0.05) according to the Tukey *post-hoc* test. RTL, root length; SHL, shoot length; NCR, number of crown root; RTW, root weight; SHW, shoot weight; TTW, total weight.

with bacteria, the colonization of plant roots by bacteria was a multistep process. We observed the colonization of bacteria in the endosphere 7 d after inoculation. At that time, the bacteria were mainly concentrated in the lateral root emergence sites, which are the root entry sites (Figure 2B). Some bacteria began to move into the root cortex. Fourteen days after inoculation, bacteria penetrated the root interior and spread throughout the roots. At that time, bacteria were concentrated in the emergent sites of the lateral roots and had moved deeper into the vascular system of the roots (Figure 2C). A similar observation was made for the G22 root system (*data not shown*).

Phosphate content profile

The Pi content in the leaves of plants grown in various Pi conditions was investigated to see how Pi was taken up in the media with and without Pi-solubilizing bacteria. The effect of the genetic background was also taken into account. In the leaves of plants, we obtained the highest Pi concentration in plants grown in the P0 medium, where it reached 5.7±0.01 and 5.025±0.03 mg/kg leaf in the G22 and G299 varieties, respectively, followed by plants grown in P+205, P+113, and P* media (Table 1). The P+ medium supplemented with the *Burkholderia* sp. 205 strain significantly increased Pi content in the leaves of plants, indicating the efficiency of this bacterial strain in dissolving insoluble Pi to soluble Pi for plant uptake. The results also showed that the low-Pi-tolerant G22 plants took up and stored more Pi in all culture conditions than the low-Pi-sensitive G299 plants.

Free amino acids content

In our study, under low Pi and full Pi media or in the medium supplemented with *Burkholderia sp*. bacteria, the free amino acid (FAA) compositions in the leaves of G299 rice plants were analyzed and are presented in Table 2.

The FAA was classified into five groups: amino acids of serine, pyruvate, aspartate, glutamate, and aromatic amino acid families (Trovato *et al.*, 2021). The results revealed that the synthesis of a few existing amino acids was highly increased under Pi starvation stress. The amino acids of the serine, aspartate, glutamate, and aromatic families were highest in G299 plants grown in the P+113 medium ($P<0.01$). In G22 plants, amino acids belonging to the serine, pyruvate, aromatic, and glutamate families were more highly synthesized in the P+113 medium (Table S2). The glutamate family was the most abundant group in G299 and G22 plants.

Table 1. The concentration of phosphate (mg/kg) in rice plants grown in various phosphate culture conditions. Different letters indicate significant differences between treatments ($P<0.05$) according to the Tukey *post-hoc* test.

	P0	P*	P+113	P+205
Rice varieties				
G22	5.70±0.11^{a}	1.41±0.02^{c}	1.52±0.01^{c}	3.81±0.04^{b}
G299	5.02±0.15^{a}	1.12±0.01^{c}	1.13±0.04^{c}	3.42±0.12^{b}

P0, full phosphate; P*, low phosphate; P+113, insoluble phosphate supplemented with *Burkholderia* sp. strain 113; P+205, insoluble phosphate supplemented with Burkholderia sp. strain 205.

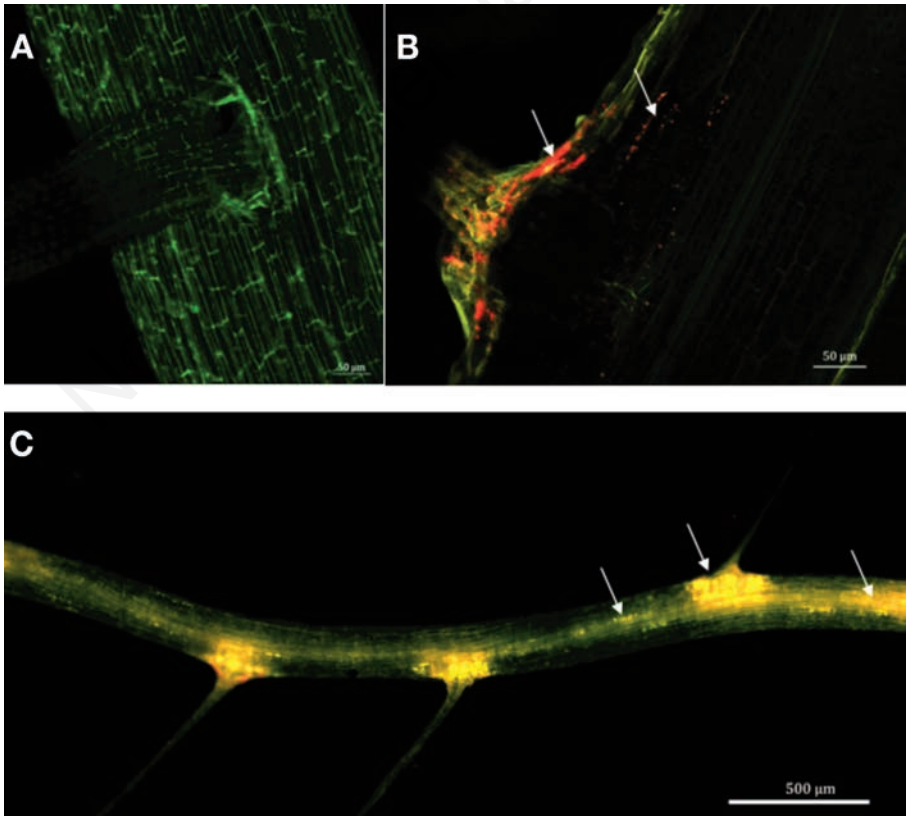


Figure 2. Confocal image of the transverse optical section of G299 rice root inoculated with *DSred*-bacteria at zero, seven days, and 14 days of inoculation. A) Root surface; B) *Dsred*-tagged bacteria were visualized at the lateral root emergence sites and cortex; C) *Dsred*-tagged bacteria were visualized at the lateral root emergence sites and vascular tissue. The white arrows indicate the bacteria's location. Scale bars: A, B) 50 m; C) 500 µm.

In the glutamate family, G299 produced significantly higher amounts of arginine than G22 plants, and glutamate was more abundant in G22 than in G299 plants in all Pi conditions (P<0.05). These differences in amino acid production indicated the different roles of amino acids in dealing with stress in G299 and G22 rice plants.

Moreover, the highest recorded total FAA was in the P+113 culture medium, reaching approximately 3.52 mg/g and 3.05 mg/g per dry leaf in the G299 and G22 varieties, respectively, followed by the P*, P+205, and P0 media (Figure 3). The total FAAs produced by plants grown in the P+113 media were 1.6 and 1.3 times higher, respectively, than that in the P0 medium in the G22 and G299 varieties. Significantly, the aromatic family content increased approximately three-fold.

The amino acids of the pyruvate and aspartate families produced by G299 and G22 rice plants grown in a low-Pi-containing medium were not significantly different (P>0.05) from those produced in a full-Pi medium, indicating that these families were not involved in response to low Pi. There was also no significant difference in threonine content in the P0 and P+205 media (P>0.05), whereas this amino acid was absent in the low-Pi-containing medium in G299. G22 plants did not produce valine.

Relative expression of the selective low-phosphate-responsive genes and jasmonic acid pathway genes in specific phosphate culture conditions

We investigated the relative expression of some essential genes (including *OsSPX1, OsCML15, OsPHO1, OsPHR2, OsPAP21,* and *OsPT9)* involved in the low-Pi-responsive pathway and *OsJAZ5* and *OsAOC* genes involved in the jasmonic acid pathway in the roots and leaves of G22 and G299 rice varieties. The results are shown in Figures 4-6. The results are depicted as the relative expression of genes from plants grown in various culture media compared to those grown in P0. In terms of the low-Pi-responsive genes, in the roots of G299 plants, the results showed the highest relative expression of all analyzed genes in the P+113 medium, followed by the P* and P+205 media. Specifically, the relative expression of the *OsPAP21* gene was approximately 140, 128, and 28 times greater in P+ 113, P*, and P+205 media, respectively, compared with the P0 medium. *OsPT9* expressed approximately

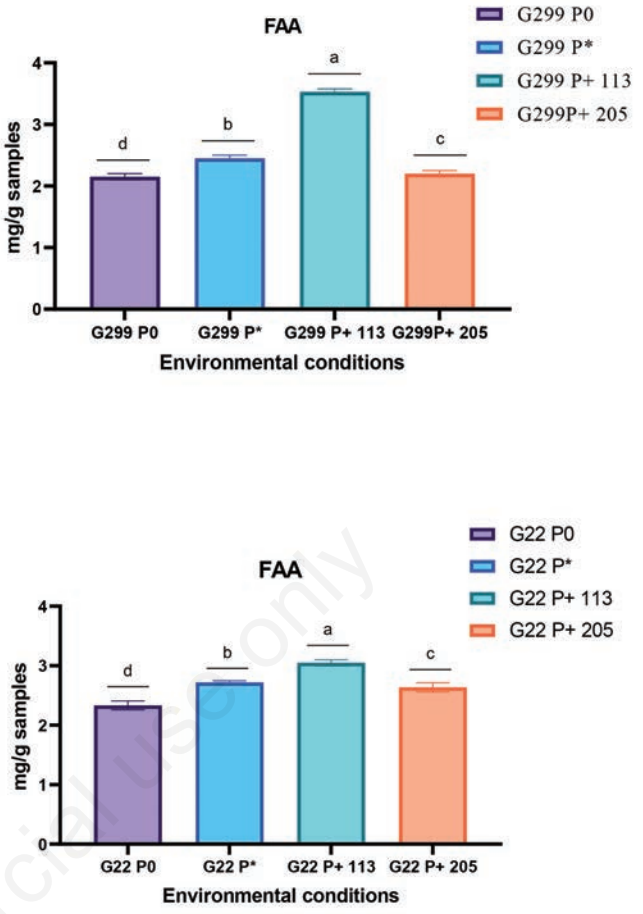


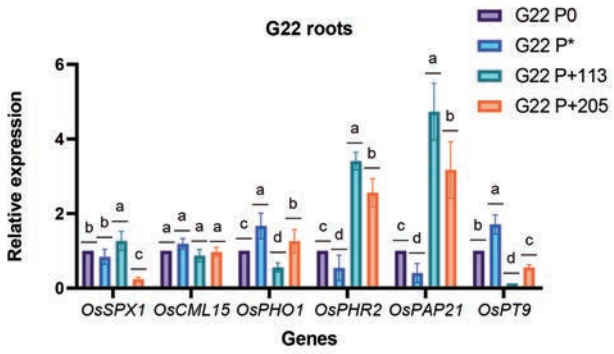
Figure 3. Effect of different phosphate applications on the concentration of total free amino acids (FAA). P0, full phosphate medium; P*, low phosphate medium; P+113, insoluble phosphate medium supplemented with *Burkholderia* sp. bacteria strain 113; P+205, insoluble phosphate medium supplemented with *Burkholderia* sp. Bacteria strain 205. Different letters indicate significant differences between treatments (P<0.05).

Table 2. Amino acid content (mg/g dry weight leaf) from leaves of G299 plants grown in different phosphate culture conditions.

Amino acids	G299 P0	G299 P*	G299 P+113	G299 P+205	Family
Serine	0.12±0.006^{b}	0.09±0.002^{c}	0.19±0.01^{a}	0.12±0.007^{b}	Serine family
Glycine	0.10±0.003^{b}	0.08±0.002^{c}	0.12±0.001^{a}	0.10±0.002^{b}	
Cysteine	0.12±0.002^{b}	0.09±0.002^{c}	0.25±0.015^{a}	0.13±0.04^{b}	
Alanine	0.36±0.01^{b}	0.38±0.02^{b}	0.52 ± 0.008^{a}	0.36±0.004^{b}	Pyruvate family
N-leucine	0.23±0.015^{a}	0.12±0.005^{b}	0.10±0.002^{c}	0.22±0.011^{a}	
Valine		0.01±0.002			
Iso-leucine	0.09±0.002^{c}	0.08±0.002^{d}	0.24±0.01^{a}	0.11±0.002^{b}	Aspartate family
Threonine	0.03±0.002^{a}			0.03±0.002^{a}	
Methionine and derivartives	0.42±0.005^{c}	0.52±0.035^{b}	0.73±0.032^{a}	0.42±0.01^{c}	
Tryptophane	0.01±0.002^{a}	0.01±0.006^{a}	0.01±0.001^{a}		Aromatic family
Tyrosine	0.06±0.003^{c}	0.05±0.002^{c}	0.19±0.009^{a}	0.08±0.001^{b}	
Phenylalanine	0.07±0.003^{c}	0.04±0.007^{d}	0.20±0.01^{a}	0.09±0.004^{b}	
Proline	0.36±0.03^{b}	0.52±0.053^{a}	0.50±0.009^{a}	0.34±0.01^{b}	Glutamic family
Arginine	0.13±0.002^{c}	0.42±0.023^{a}	0.38±0.015^{b}	0.13±0.007^{c}	
Histidine	0.04±0.004^{b}	0.03±0.001^{c}	0.07±0.001^{a}	0.05±0.005^{b}	
Glutamine	0.02±0.004^{a}	0.01±0.002^{c}	0.02±0.003^{a}	0.02±0.006^{a}	

P0, full phosphate; P*, low phosphate; P+113, unsoluble phosphate supplemented with *Burkholderia* sp. strain 113; P+205, unsoluble phosphate supplemented with *Burkholderia* sp. strain 205. Different letters indicate significant differences between treatments (P<0.05) according to the Tukey *post-hoc* test.

56, 42, and 40 times more in the roots of G299 plants grown in the P+113, P*, and P+205 media than those in the P0 medium, respectively (Figure 4). In contrast, a much lower relative expression of these genes was observed in the roots of low-Pi-tolerant G22 rice plants (Figure 5). The highest relative expression was also obtained in plants grown in the P+113 medium, where we obtained approximately five and three times greater relative expression of *OsPAP21* and *OsPHR2* genes, respectively, than in plants grown in the P0 medium. In the leaves of the G299 variety, except for the *OsSPX1* gene, we also obtained the highest expression in the plants grown in the P+113 medium, at approximately seven times more than those grown in the P0 medium. All other studied genes were expressed at very low levels. A very low relative expression level of five out of the six investigated genes was observed in the leaves of low-Pi-tolerant G22 rice plants. Only *OsSPX1* expressed approximately three times higher in plants grown in P+113 than in the P0 medium (Figure 5).



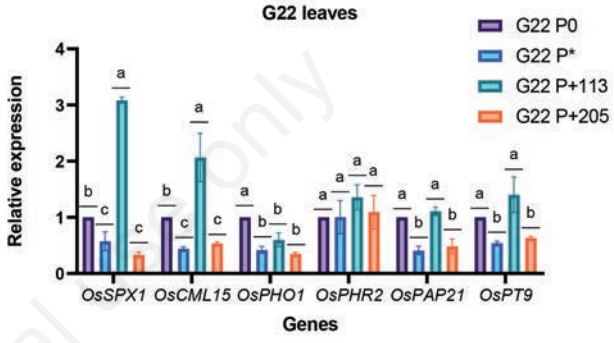
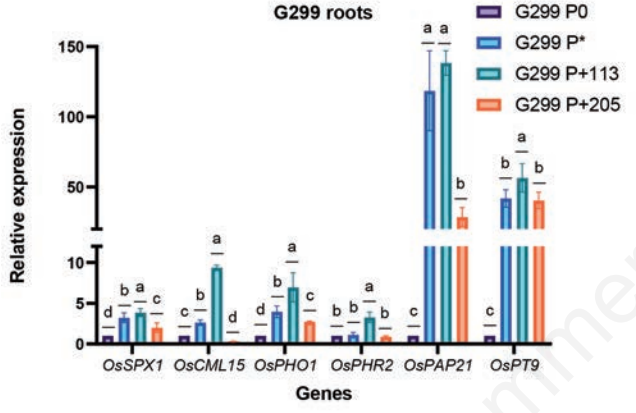


Figure 5. Relative expression of genes in the G22 rice variety grown in different culture media. The relative gene expression is calculated in various conditions against the P0 condition. P0, full phosphate; P*, low phosphate; P+113, insoluble phosphate supplemented with *Burkholderia* sp. strain 113; P+205, insoluble phosphate supplemented with *Burkholderia* sp. strain 205. Different letters indicate significant differences between treatments (P<0.05). The data were expressed as the mean of three biological replications ± standard deviation.



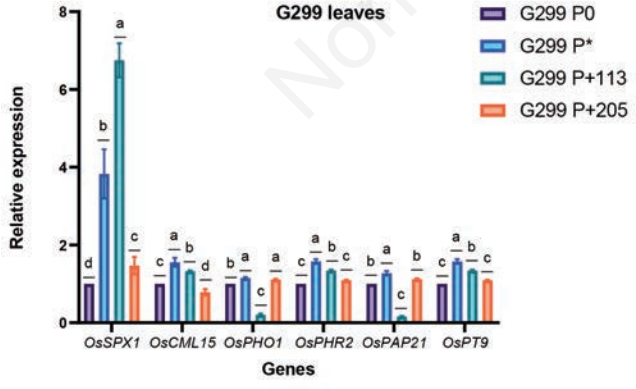


Figure 4. Relative expression of genes in the G299 rice variety grown in different culture media. The relative gene expression is calculated in various conditions against the P0 condition. P0, full phosphate; P*, low phosphate; P+113, insoluble phosphate supplemented with *Burkholderia* sp. strain 113; P+205, insoluble phosphate supplemented with *Burkholderia* sp. strain 205. The data were expressed as the mean of three biological replications ± standard deviation. Letters indicate a significant difference (P<0.05).

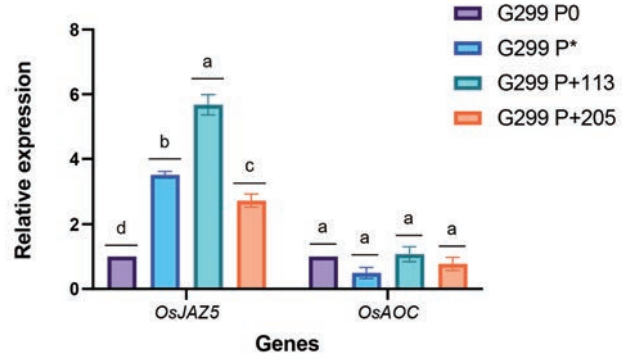


Figure 6. Relative expression of jasmonic acid pathway genes in the G299 rice variety grown in different culture media. The relative gene expression is calculated in various conditions against the P0 condition. P0, full phosphate; P*, low phosphate; P+113, insoluble phosphate supplemented with *Burkholderia* sp. strain 113; P+205, insoluble phosphate supplemented with *Burkholderia* sp. strain 205. The data were expressed as the mean of three biological replications ± standard deviation. Letters indicate a significant difference (P<0.05).

Regarding the jasmonic acid pathway, we observed a significant increase in the expression of the *OsJAZ5* gene in the roots of the low-phosphate-sensitive G299 rice variety grown in a low Pi medium. Specifically, the relative expression of the *OsJAZ5* gene was approximately 3, 3.5, and 4.5 times greater in P+205, P*, and P+113 media, respectively, compared with the P0 medium (Figure 6). No significant difference was obtained in the expression of *OsJAZ5* and *OsAOC* genes in the shoots of both the low-phosphate-sensitive G299 rice variety and the low-phosphate-tolerant G22 rice variety or the roots of the G22 variety (P>0.05) (*data not shown*).

Discussion

Phosphate is an essential element for plant development and a limiting source. Under Pi-limiting conditions, especially during the vegetative stage, plants require very high nutrients. Hence, they have developed multiple strategies, including morphological, biochemical, and molecular approaches to meet these requirements. In this study, we investigated the response strategies of two contrasting rice plants to the limited availability of Pi supplemented with endophytic *Burkholderia sp*. 113 and 205 strains at the seedling stage. The workflow of this study is presented in Figure S2. However, further studies about the pathogenic effects of *Burkholderia* bacteria on rice plants, other crops, and the environment need to be carefully investigated before deciding whether or not to use it as a biofertilizer.

Phenotypic response to low phosphate conditions

The results showed a significant increase in RTL and RTW of rice varieties grown in low-Pi media (P* and P+113) than in high-Pi media (P0 and P+205). This phenomenon indicated that rice plants strongly developed their root systems under the Pi-deficient-medium to obtain and take up more Pi. This was also observed in other studies, where the root system is highly responsive to environmental changes to explore different soil layers to forage for nutrients (Crombez *et al.*, 2019). Plants can develop lateral roots and root hairs (Jiang *et al.*, 2007), inhibit primary root growth (López-Bucio *et al.*, 2003) or increase the root-to-shoot ratio (Lynch and Brown, 2001; López-Bucio *et al.*, 2003). However, SHW and SHL were lower in plants grown in P0 and P+205 media than in those grown in P* and P+113 media. Similarly, a considerable reduction in the growth of shoots, roots, and biomass was observed during the vegetative stage of rice plants (Kumar *et al.*, 2021).

The low-Pi-tolerant G22 and low-Pi-sensitive G299 genotype responded differently to the Pi starvation condition due to the different genetic backgrounds. We observed a lower reduction of SHW in the G22 variety than in the G299 variety and a higher increase in RTL and RTW in variety G299 than in G22 when plants were grown in low-Pi-containing media, showing the involvement of genetic background in response to the low Pi medium. The different genetic backgrounds also resulted in differing responses to low Pi in the study by Kumar *et al*. (2021), where an approximately 50% reduction in length and 72% reduction in the width of the shoots was observed in the low-P-sensitive genotype (Pusa-44). In contrast, the drop was 41-44% in the low-Pi-tolerant genotypes (NIL-23 and Kasalath) (Kumar *et al.*, 2021).

The plants grown in the P+205 medium increased SHL and SHW and reduced RTL, RTW, and NCR to a greater extent than the plants grown in the P+113 medium. These results showed the correlation between the solubilization of the *Burkholderia* strain and the improved growth of rice plants. The efficiency of *Burkholderia* sp. strain 205 in dissolving insoluble Pi $Ca_3(PO_4)_2$ has been investigated *in vitro* in our previous study. In the present study, we have confirmed the efficiency of this strain *in vivo* in two investigated rice varieties.

Colonization of endophytic bacteria in roots

We observed that the colonization of *Burkholderia* sp. is a multiple-step process, where the bacteria start at the lateral root emergence sites, then penetrate the root interior in the vascular system of the roots, and spread everywhere inside the roots. A similar observation was observed with *Burkholderia* sp. in grapevines (Compant *et al.*, 2008). In another study, *Paraburkholderia kururiensis* primarily colonized root hairs and then spread into xylem vessels in rice plants (Mattos *et al.*, 2008). The colonization process of bacteria in root plants can be either active or passive. Plant hosts also play a critical role in endophytes (Afzal *et al.*, 2019).

Biochemical response to low phosphate conditions

We observed the efficiency of *Burkholderia* sp. 205 in dissolving insoluble Pi into soluble Pi, resulting in a higher Pi concentration accumulated in the shoots of plants grown in P+205 than in P+113 media in both G22 and G299 plants. The mechanism for solubilization of insoluble phosphate can primarily be explained by organic acid production, which reduces pH, then H^+ replaces Ca^{2+} to release the phosphate ion (Chhabra and Dowling, 2017; Alori *et al*., 2017). Moreover, acid phosphatases and enzyme phytases secreted by these endophytic bacteria also make Pi available to plants (Mei *et al*., 2021). The results also showed that the low-Pi-tolerant G22 plants took up and stored more Pi in all culture conditions than the low-Pi-sensitive G299 plants. These results can be explained by the genetic divergence of the two rice varieties, which belong to the *Indica* and the *Japonica* groups. A better Pi acquisition by the low-Pi-tolerant rice genotype resulted in a higher Pi concentration in the leaves of this genotype than in the leaves of the low-Pi-sensitive genotype (Kumar *et al.*, 2021). A similar phenomenon was also obtained in terms of nitrogen-use efficiency. A higher nitrogen-use efficiency was obtained in the *Indica* variety than in the *Japonica* variety (Zhang *et al*., 2019).

The plants also synthesized significantly more amino acids of the serine, aspartate, glutamate, and aromatic families when grown in a P+113 medium. The involvement of amino acids in stress tolerance has been demonstrated to affect the synthesis and activities of some enzymes, gene expression, and redox homeostasis (Liu *et al*., 2010). The increased synthesis of some amino acids may also be related to the production of secondary metabolites and signaling molecules to cope with stress (Batista-Silva *et al.,* 2019). Some amino acids serve as precursors for the synthesis of secondary metabolites. For example, methionine, alanine, branched-chain, and aromatic amino acids are precursors of glucosinolates (Halkier and Gershenzon, 2006). The amino acid proline is considered especially important because it is believed to contribute to stress tolerance in rice (Sabbioni *et al.*, 2021) and *Arabidopsis* (Székely *et al.*, 2008). The aromatic family is involved in salt stress, and the plant parasite *Phelipanche aegyptica* tolerance in tobacco suggests that increasing this amino acid family can effectively combat plant parasites (Oliva *et al.*, 2021). Moreover, the total FAA was also highest in plants grown in the stress P+113 medium because this medium accumulated the highest stress level without access to soluble Pi. Similarly, the total FAA concentration was the highest in potatoes in the low-Pi-containing medium, which increased amino acids by 1.5–14.8 times (Chea *et al.*, 2021). Asparagine demonstrated the greatest increase at 14.8 times. Increasing Pi application

to the culture medium decreased total amino acid and proline levels (Chea *et al.*, 2021). In the shoots of barley, severe Pi deficiency led to increased concentrations of half of the amino acids examined, especially glutamine and asparagine (Huang *et al.*, 2008). Furthermore, the amino acid profile was different between the G22 and G299 rice varieties in response to low Pi, especially in the glutamate family, showing the effect of genetic background on producing amino acids in response to Pi stress.

The results from these morphophysiological and biochemical studies suggest that the alteration of amino acids to enable more efficient utilization of Pi is a valid strategy for improving the ability of rice plants to adapt to low Pi environments.

Molecular response to low phosphate conditions

In addition to morphological and biochemical adaptations, plants use molecular approaches to respond to low Pi conditions by modulating gene expression. The qPCR analysis of six low-Pi-responsive genes, including *OsSPX1, OsCML15, OsPHO1, OsPHR2, OsPAP21,* and *OsPT9,* in two contrasting rice genotypes, revealed that all six genes were strongly upregulated in the roots of the G299 variety to deal with low-Pi-media. The six selected genes were involved in low-Pi-responsive pathways. The increase in their expression under low Pi conditions has also been reported in other studies (Zhou *et al.*, 2008; Li *et al.*, 2010; Deng *et al.*, 2022). Significantly, the *OsPAP21* gene expression increased approximately 140 times in plants grown in the P+113 medium compared to those grown in the P0 medium. The expression of *OsPT9* was approximately 56 times greater in the roots of G299 plants grown in the P+113 than those grown in the P0 medium. These results indicated that under a Pi starvation medium supplemented with bacteria, the low-Pi-sensitive G299 rice plants activated the highest low-Pi-responsive genes to find and transport more Pi. However, a much lower relative expression of these six genes was observed in the roots of low-Pi-tolerant G22 rice plants. In the leaves of varieties G22 and G299, except the *OsSPX1* gene, all other studied genes were expressed at very low levels. Our results indicate that roots play a more critical role in Pi starvation than leaves. Our findings agree with Li *et al*. (2010) and Deng *et al*. (2022), where Dongxiang and Lagrue rice varieties upregulated the expression of Pi transporters *OsPTs* and acid phosphatase *OsPAPs* in roots. The upregulation of the *OsPTs* gene is responsible for Pi acquisition from the soil and mobilization of Pi within plants (Shin *et al.*, 2004). The secretion of acid phosphatase helps release Pi and organic phosphate in crops (Liang *et al.*, 2014). Specific induction of *OsSPX1* was observed in both the leaves and roots of rice plants under Pi starvation (Wang *et al.*, 2009; Deng *et al.*, 2018). Our results also indicated the different roles of genes involved in the low-Pi-responsive pathway in the different plant organisms.

We demonstrated the involvement of the *OsJAZ5* gene, a gene in the jasmonic synthesis pathway, through significantly increased expression in the roots of the low-phosphate-sensitive G299 rice variety in response to the low Pi condition ($P<0.001$). We observed significantly increased expression of the *OsJAZ5* gene in the roots ($P<0.001$) but not in the shoots of the low-phosphate-sensitive G299 rice variety and not in the roots and shoots of the low-phosphate-tolerant-G22 variety ($P>0.05$). These results showed that the sensitive variety not only activated the low-Pi-responsive genes to deal with low Pi stress but also activated the jasmonic acid gene, which plays an essential role in mitigating biotic and abiotic stresses in plants. The involvement of jasmonic acid genes in dealing with Pi starvation has also been shown in other studies where 15 *OsJAZ* genes, including the *OsJAZ5* gene, were found to be strongly activated in rice (Singh AP *et al*, 2015), sorghum (Zhang *et al*., 2019) and cotton (cv. YZ1) (Luo *et al*., 2021). Furthermore, for the first time, the culture medium supplemented with Pi-solubilizing-bacteria *Burkholderia* sp. 205 lowered the expression of the *OsJAZ5* gene in the P+205 medium compared with the low-Pi-containing medium (P* or P+113 medium). Another jasmonic acid-related gene named *OsJAZ1* was also downregulated in *Enterobacter ludwigii* GAK2-inoculated Korean Hwayeongbyeo rice plants where *Enterobacter ludwigii* GAK2 acted as a Pi-solubilizing-bacteria (Adhikari *et al*., 2020). The study conducted by King *et al.*, (2019) demonstrated a temporal shift in jasmonic acid systemic response in Nipponbare rice plants inoculated with *Burkholderia vietnamiensis* and *Paraburkholderia kururiensis* where *JAZ6, JAZ10, JAZ12, ATL15*, and *AOS1* were upregulated when plants were inoculated with *Paraburkholderia kururiensis*; and *JAZ6* and *JAZ10* were down-regulated in response to *Burkholderia vietnamiensis*.

Conclusions

The current study reports a significant difference in the effect of *Burkholderia sp*. strains 113 and 205 as well as the role of the genetic background of rice plants on Pi use efficiency, thus facilitating research on improving Pi utilization efficiencies in rice plants. Furthermore, for the first time, we showed a change in the expression of *OsJAZ5* in the rice plants grown in the insoluble Pi-containing medium supplemented with *Burkholderia sp*. strains 205. Significantly higher RTL, RWT, and NCR were observed in P* and P+113 media than in P0 and P+205 media, but SHW was reversed in both rice varieties. Four of the five amino acid families and the total free amino acids were significantly higher in the P+113 medium than in the others. The relative expression of all Pi-investigated genes was highest in the P+113 medium in the low-Pi-sensitive G299 variety roots. A promising strategy can be proposed from our study for crop plants to adapt to abiotic stresses by taking advantage of the flexibility of biodiversity in the genetics of species.

References

Adhikari A, Lee KE, Khan MA, Kang SM, Adhikari B, Imran M, Jan R, Kim KM, Lee IJ, 2020. Effect of silicate and phosphate solubilizing rhizobacterium enterobacter ludwigii GAK2 on oryza sativa L. under cadmium stress. J Microbiol. Biotechnol. 30:118-26.

Afzal I, Shinwari ZK, Sikandar S, Shahzad S, 2019. Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. Microbiol. Res. 221:36-49.

Alori ET, Glick BR, Babalola OO, 2017. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front. Microbiol. 8:971.

Babalola OO, Bernard RG, 2012. The use of microbial inoculants in African agriculture: current practice and future prospects. J. Food Agric. Environ. 10:540-9.

Baqual MF, Das PK, 2006. *I*nfluence of Biofertilizers on Macronutrient uptake by the Mulberry Plant and its Impact on Silkworm Bioassay. Caspian J. Env. Sci. 4:98-109.

Bargaz A, Elhaissoufi W, Khourchi S, Benmrid B, .Borden KA, Rchiad Z, 2021. Benefits of phosphate solubilizing bacteria on belowground crop performance for improved crop acquisition of phosphorus. Microbiol. Research. 252:126842.

Barra PJ, Viscardi S, Jorquera MA, Duran PA, Valentine AJ, de la Luz Mora M, 2018. Understanding the strategies to overcome phosphorus–deficiency and aluminum–toxicity by ryegrass endophytic and rhizosphere phosphobacteria. Front. Microbiol. 9:1155.

Batista-Silva W, Heinemann B, Rugen N, Nunes-Nesi A, Araújo WL, Braun HP, Hildebrandt TM, 2019. The role of amino acid metabolism during abiotic stress release. Plant Cell Env. 42:1630-44.

Bechtaoui N, Rabiu MK, Raklami A, Oufdou K, Hafidi M, Jemo M, 2021. Phosphate-dependent regulation of growth and stresses management in plants. Front. Plant Sci. 12:2357.

Chhabra S, Dowling DN, 2017. Endophyte-Promoted Nutrient Acquisition: Phosphorus and Iron. In: Doty S. (eds). Functional Importance of the Plant Microbiome. Springer Cham. pp. 21-4.

Charana Walpola B, 2012. Prospectus of phosphate solubilizing microorganisms and phosphorus availability in agricultural soils: a review increase the dissolution rate of eppawala rock phosphate (erp) by using phosphate solubilizing microorganisms. African J. Microbiol. 6:6600-5.

Chea L, Pfeiffer B, Schneider D, Daniel R, Pawelzik E, Naumann M, 2021. Morphological and metabolite responses of potatoes under various phosphorus levels and their amelioration by plant growth-promoting rhizobacteria. Int. J. Mol. Sci. 22:5162.

Chen Q, Liu S, 2019. Identification and characterization of the phosphate-solubilizing bacterium pantoea sp. S32 in reclamation soil in Shanxi, China. Front. Microbiol. 10:2171.

Compant S, Kaplan H, Sessitsch A, Nowak J, Ait Barka E, Clément C, 2008. Endophytic colonization of Vitis vinifera L. by Burkholderia phytofirmans strain PsJN: from the rhizosphere to inflorescence tissues. FEMS Microbiol. Ecol. 63:84–93.

Crombez H, Motte H, Beeckman T, 2019. Tackling Plant Phosphate Starvation by the Roots. Dev. Cell 48:599-615.

Deng Q, Dai L, Chen Y, Wu D, Shen Y, Xie J, Luo X, 2022. Identification of phosphorus stress related proteins in the seedlings of dongxiang wild rice (oryza rufipogon griff.) using label-free quantitative proteomic analysis. Genes.13:108.

Deng QW, Luo XD, Chen YL, Zhou Y, Zhang FT, Hu BL, Xie JK, 2018. Transcriptome analysis of phosphorus stress responsiveness in the seedlings of dongxiang wild rice (oryza rufipogon griff.). Biol. Res. 51:1-12.

Ezawa T, Smith SE, Smith FA, 2002. P metabolism and transport in AM fungi. Plant Soil 244:221-30.

Haefele SM, Nelson A, Hijmans RJ, 2014. Soil quality and constraints in global rice production. Geoderma 235–236:250–9.

Halkier BA, Gershenzon J, 2006. Biology and Biochemistry of Glucosinolates. Annu. Rev. Plant Biol. 57:303-33.

Hirsch J, Marin E, Floriani M, Chiarenza S, Richaud P, Nussaume L, Thibaud MC, 2006. Phosphate deficiency promotes modification of iron distribution in Arabidopsis plants. Biochimie. 88:1767-71.

Huang CY, Roessner U, Eickmeier I, Genc Y, Callahan DL, Shirley N, Langridge P, Bacic A, 2008. Metabolite profiling reveals distinct changes in carbon and nitrogen metabolism in phosphate-deficient barley plants (Hordeum vulgare L.). Plant Cell Physiol. 49:691-703.

Jia X, Ye J, Wang H, Li L, Wang F, Zhang Q, Chen J, Zheng X, He H, 2018. Characteristic amino acids in tea leaves as quality indicator for evaluation of Wuyi Rock Tea in different cultured regions. J. Appl. Bot. Food Qual. 91:187-93.

Jiang C, Gao X, Liao L, Harberd NP, Fu X, 2007. Phosphate starvation root architecture and anthocyanin accumulation responses are modulated by the gibberellin-DELLA signaling pathway in arabidopsis. Plant Physiol. 145:1460-70.

Jilani G, Akram A, Ali RM, Hafeez FY, Shamsi IH, Chaudhry AN, Chaudhry AG, 2007. Enhancing crop growth, nutrients availability, economics and beneficial rhizosphere microflora through organic and biofertilizers. Ann. Microbiol. 57:177-84.

Kariman K, Barker SJ, Finnegan PM, Tibbett M, 2014. Ecto- and arbuscular mycorrhizal symbiosis can induce tolerance to toxic pulses of phosphorus in jarrah (Eucalyptus marginata) seedlings. Mycorrhiza 24:501-9.

King E, Wallner A, Rimbault E, Barrachina C, Klonowska A, Moulin L, Czernic P, 2019. Monitoring of rice transcriptional responses to contrasted colonizing patterns of phytobeneficial burkholderia s.l. reveals a temporal shift in ja systemic response. Front. Plant Sci. 10:1141.

Kirkby EA, Johnston AE, 2008. Soil and fertilizer phosphorus in relation to crop nutrition.In: White PJ, Hammond JP (eds). The ecophysiology of plant-phosphorus interactions. Plant ecophysiology, vol 7. Dordrecht: Springer. pp. 177-223.

Kobayashi T, Itai RN, Senoura T, Oikawa T, Ishimaru Y, Ueda M, Nakanishi H, Nishizawa NK, 2016. Jasmonate signaling is activated in the very early stages of iron deficiency responses in rice roots. Plant Mol. Biol. 91:533-47.

Kumar S, Pallavi, Chugh C, Seem K, Kumar S, Vinod KK, Mohapatra T, 2021. Characterization of contrasting rice (Oryza sativa L.) genotypes reveals the Pi-efficient schema for phosphate starvation tolerance. BMC. Plant Biol. 21:282.

Li L, Liu C, Lian X, 2010. Gene expression profiles in rice roots under low phosphorus stress. Plant Mol. Biol. 72:423-32.

Liang C, Wang J, Zhao J, Tian J, Liao H, 2014. Control of phosphate homeostasis through gene regulation in crops. Curr. Opin. Plant Biol. 21:59-66.

Lindsay WL, 1979. Chemical equilibria in soils. Clays Clay Miner. 28:319.

Liu G, Ji Y, Bhuiyan NH, Pilot G, Selvaraj G, Zou J, Wei Y, 2010. Amino acid homeostasis modulates salicylic acid–associated redox status and defense responses in arabidopsis. Plant Cell. 22:3845.

Livak KJ, Schmittgen TD, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 25:402-8.

López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L, 2003. The role of nutrient availability in regulating root architecture. Curr. Opin. Plant Biol. 6:280-7.

Luo X, Li Z, Xiao S, Ye Z, Nie X, Zhang X, Kong J, Zhu L, 2021. Phosphate deficiency enhances cotton resistance to Verticillium dahliae through activating jasmonic acid biosynthesis and phenylpropanoid pathway. Plant Sci. 302:110724.

Lynch JP, Brown KM, 2001. Topsoil foraging – an architectural adaptation of plants to low phosphorus availability. Plant Soil 237:225-37.

Mai NTP, Mai CD, Nguyen H Van, Le KQ, Duong LV, Tran TA, To HTM, 2021. Discovery of new genetic determinants of morphological plasticity in rice roots and shoots under phosphate starvation using GWAS. J. Plant Physiol. 257:153340.

Mattos KA, Pádua VLM, Romeiro A, Hallack LF, Neves BC, Ulisses TMU, Barros CF, Todeschini AR, Previato JO, Mendonça-Previato L, 2008. Endophytic colonization of rice (Oryza sativa L.) by the diazotrophic bacterium Burkholderia kururiensis and its ability to enhance plant growth. An. Acad. Bras. Cienc. 80:477-93.

Mei C, Chretien RL, Amaradasa BS, He Y, Turner A, Lowman S,

2021. Characterization of phosphate solubilizing bacterial endophytes and plant growth promotion in vitro and in greenhouse. Microorganisms 9:1935.

Oliva M, Guy A, Galili G, Dor E, Schweitzer R, Amir R, Hacham Y, 2021. Enhanced production of aromatic amino acids in tobacco plants leads to increased phenylpropanoid metabolites and tolerance to stresses. Front. Plant Sci. 11:2110.

Paz-Ares J, Puga MI, Rojas-Triana M, Martinez-Hevia I, Diaz S, Poza-Carrión C, Miñambres M, Leyva A, 2022. Plant adaptation to low phosphorus availability: core signaling, crosstalks, and applied implications. Mol. Plant 15:104-24.

Raj DP, 2014. Molecular characterization of Phosphate Solubilizing Bacteria (PSB) and Plant Growth Promoting Rhizobacteria (PGPR) from pristine soils. Int. J. Innov. Sci. Eng. Technol. 1:317-24.

Sabbioni G, Funck D, Forlani G, 2021. Enzymology and regulation of δ1-Pyrroline-5-carboxylate synthetase 2 from rice. Front. Plant Sci. 12:1894.

Secco D, Baumann A, Poirier Y, 2010. Characterization of the rice PHO1 gene family reveals a key role for OsPHO1;2 in phosphate homeostasis and the evolution of a distinct clade in dicotyledons. Plant Physiol. 152:1693-704.

Singh AP, Pandey BK, Deveshwar P, Narnoliya L, Parida SK, Giri J, 2015. JAZ repressors: potential involvement in nutrients deficiency response in rice and chickpea. Front. Plant Sci. 6:975.

Shin H, Shin HS, Dewbre GR, Harrison MJ, 2004. Phosphate transport in arabidopsis: Pht1;1 and Pht1; 4 play a major role in phosphate acquisition from both low- and high-phosphate environments. Plant J. 39:629-42.

Stephen J, Shabanamol S, Rishad KS, Jisha MS, 2015. Growth enhancement of rice (Oryza sativa) by phosphate solubilizing Gluconacetobacter sp. (MTCC 8368) and Burkholderia sp. (MTCC 8369) under greenhouse conditions. 3 Biotech. 5:831-7.

Székely G, Ábrahám E, Cséplo Á, Rigó G, Zsigmond L, Csiszár J, Ayaydin F, Strizhov N, Jásik J, Schmelzer E, Koncz C, Szabados L, 2008. Duplicated P5CS genes of arabidopsis play distinct roles in stress regulation and developmental control of proline biosynthesis. Plant J. 53:11-28.

Tajini F, Trabelsi M, Drevon JJ, 2012. Combined inoculation with glomus intraradices and Rhizobium tropici CIAT899 increases phosphorus use efficiency for symbiotic nitrogen fixation in common bean (Phaseolus vulgaris L.). Saudi J. Biol. Sci. 19:157-63.

Tawaraya K, 2022. Response of mycorrhizal symbiosis to phosphorus and its application for sustainable crop production and remediation of environment. Soil Sci. Plant Nutr. 68:1-5.

To TMH, Nguyen TH, Dang TMN, Nguyen HN, Bui XT, Lavarenne J, Phung TPN, Gantet, P, Lebrun M, Bellafiore S, Champion A, 2019. Unraveling the genetic elements involved in shoot and root growth regulation by jasmonate in rice using a genome-wide association study. Rice (N Y). 12:69.

To HTM, Le KQ, Van Nguyen H, Duong LV, Kieu HT, Chu QAT, Tran TP, Mai NTP, 2020. A genome-wide association study reveals the quantitative trait locus and candidate genes that regulate phosphate efficiency in a Vietnamese rice collection. Physiol. Mol. Biol. Plants 26:2267-81.

Trovato M, Funck D, Forlani G, Okumoto S, Amir R, 2021. Editorial: amino acids in plants: regulation and functions in development and stress defense. Front. Plant Sci. 12:2287.

Varga T, Hixson KK, Ahkami AH, Sher AW, Barnes ME, Chu RK, Battu AK, Nicora CD, Winkler TE, Reno LR, Fakra SC, Antipova O, Parkinson DY, Hall JR, Doty SL, 2020. Endophyte-promoted phosphorus solubilization in populus. Front. Plant Sci. 11:1585.

Wang X, Wang Y, Piñeros MA, Wang Z, Wang W, Li C, Wu Z, Kochian L V., Wu P, 2014. Phosphate transporters OsPHT1; 9 and OsPHT1; 10 are involved in phosphate uptake in rice. Plant. Cell Environ. 37:1159-70.

Wang Z, Xu G, Ma P, Lin Y, Yang X, Cao C, 2017. Isolation and characterization of a phosphorus-solubilizing bacterium from rhizosphere soils and its colonization of Chinese cabbage (Brassica campestris ssp. chinensis). Front. Microbiol. 8:1270.

Wang C, Ying S, Huang H, Li K, Wu P, Shou H, 2009. Involvement of OsSPX1 in phosphate homeostasis in rice. Plant J. 57:895–904.

Wu P, Ma L, Hou X, Wang M, Wu Y, Liu F, Deng XW, 2003. Phosphate starvation triggers distinct alterations of genome expression in arabidopsis roots and leaves. Plant Physiol. 132:1260-71.

Yoshida S, Forno DA, Cock J, 1971. Laboratory Manual for Physiological Studies of Rice. Available from: http://books.irri.org/9711040352_content.pdf.

Zhang J, Liu YX, Zhang N, Hu B, Jin T, Xu H, Qin Y, Yan P, Zhang X, Guo X, Hui J, Cao S, Wang X, Wang C, Wang H, Qu B, Fan G, Yuan L, Garrido-Oter R, Chu C, Bai Y, 2019a. NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. Nat Biotechnol. 37:676-84.

Zhang J, Jiang F, Shen Y, Zhan Q, Bai B, Chen W, Chi Y, 2019b. Transcriptome analysis reveals candidate genes related to phosphorus starvation tolerance in sorghum. BMC Plant Biol. 19:1-18.

Zhang Q, Wang C, Tian J, Li K, Shou H, 2011. Identification of rice purple acid phosphatases related to phosphate starvation signalling. Plant Biol. (Stuttg). 13:7-15.

Zhou J, Jiao FC, 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 Physiol. 146:1673-86.