

Overexpression of *OsMAPK2* Enhances Low Phosphate Tolerance in Rice and *Arabidopsis thaliana*

Yeon Jae Hur, Doh Hoon Kim*

College of Natural Resources and Life Science, Dong-A University, Busan, South Korea.
Email: dhkim@dau.ac.kr

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ABSTRACT

The mitogen-activated protein kinase (MAPK) cascade is the most important mechanism in environmental responses and developmental processes in plants. The *OsMAPK2* gene has been found to function in plant tolerance to diverse biotic/abiotic stresses. This paper presents evidence that *OsMAPK2* (*Oryza sativa* MAP kinase gene 2) is responsive to P_i deficiency and involved in P_i homeostasis. We found that full-length expression of *OsMAPK2* was up-regulated in both rice plants and cell culture in the absence of inorganic phosphate (P_i). The transgenic rice and *Arabidopsis* plants overexpressing *OsMAPK2* showed affected root development and increased plant P_i content compared with wild-type plants. Overexpression of *OsMAPK2* controlled the expression of several P_i starvation-responsive genes. Our results indicated that *OsMAPK2* enables tolerance phosphate deficiency and is involved in P_i homeostasis.

KEYWORDS

Rice (*Oryza sativa*); Phosphorylation; MAP Kinase; Phosphate; Homeostasis

1. Introduction

Rice is a monocot model plant, as well as an important cereal crop that supplies food for more than half of the world population. Rice (*Oryza sativa* L.) is also a model organism to study functional genomics of monocot plants because its genome size is smaller than those of other monocot plants.

Phosphate (P_i) is one of the most important nutrients for plant growth and development, but the availability of phosphate is frequently a limiting factor for crop productivity. This is because plants preferentially take up P_i as orthophosphate, and more than 80% of soil P_i is immobilized and is not readily available to roots [1,2]. Plants respond to P_i deficiency by activating mechanisms to increase P_i bioavailability and uptake. In response to persistent P_i deficiency, plants have developed many adaptive mechanisms to enhance availability and increase the uptake of P_i , and plants exhibit morphological modifications and biochemical changes as well as regulate the

expression of genes in response to cues in the phosphate signaling pathway [2,3]. Secreting acid phosphatase increases P_i bioavailability by cleaving phosphate groups esterified to organic acids [4-6]. Although some of the components of P_i -starvation signaling in plants have been identified, the overall pathways are still poorly understood. It is important to study the functions of phosphate deficient condition responsive genes to increase the productivity and distribution of crop plants.

One of the major pathways by which external stimuli are transduced into intracellular responses is the mitogen-activated protein kinase (MAPK) signaling pathway [7,8] (Figure 1). MAPK cascades are composed of three protein kinase modules: MAPKK kinases (MAPKKKs), MAPK kinases (MAPKKs) and MAPKs, which are linked in various ways to upstream receptors and downstream targets. In this phosphorylation module, a MAPKKK phosphorylates a MAPKK. MAPKKs are activated when serine and serine/threonine residues in the S/T-X3-5-S/T motif are phosphorylated by serine/threonine kinases MAPKKKs [9]. MAPK is then activated by the dual-

*Corresponding author.

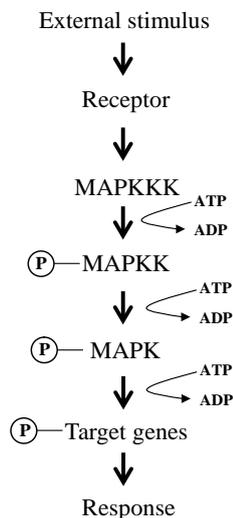


Figure 1. Map kinase cascade. Details are mentioned in the text.

specificity kinase MAPKK. A dual phosphorylation activation motif of MAPK is threonine and tyrosine residues in a TXY located between subdomains VII and VIII [10]. Activated MAPK is often imported into the nucleus, where it phosphorylates and activates specific downstream signaling components, such as transcription factors [11]. The activated specific MAP kinases phosphorylate various downstream targets and regulate stress and hormonal responses, innate immunity, and developmental programs [12-15].

To date, most of the reported plant MAPKs have been isolated and characterized from dicot model species such as *Arabidopsis* and tobacco. *Arabidopsis AtMPK3* kinase expresses strongly after cold, touch and dehydration in terms of transcription level [16]. Some MAPKs have also been identified and characterized from rice (*Oryza sativa*; [17-21]).

The *OsMAPK2* gene (also known as *OsMSRMK2*, *Os-BIMK1*, or *OsMAP1*) has been reported, and it was demonstrated that *OsMAPK2* may play a cellular role in various biotic and abiotic stresses [18-22].

In this study, we obtained transgenic rice overexpressing the full-length *OsMAPK2* gene and reported the effect of *OsMAPK2* in response to phosphate deficient conditions and the acquisition of P_i . These results reveal that *OsMAPK2* regulates the expression of an important set of genes in response to phosphate deficient conditions.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

Rice (*Oryza sativa* L. cv. Dongjin) plants were grown in a hydroponic growth facility with one-half strength Hoagland solution. Phosphorus starvation treatments

were initiated three weeks after germination. The plants were transferred to an aerated hydroponics solution [5 mM $Ca(NO_3)_4 \cdot 4H_2O$, 2.5 mM K_2SO_4 , 2 mM $MgSO_4 \cdot 7H_2O$, 500 μM KH_2PO_4 , 0.12 mM KCl, 0.25 M $MnSO_4$, 0.25 M H_3BO_3 , 0.25 M $ZnSO_4$, 20 mM $CuSO_4$, 20 mM Na_2MoO_4 , 9.8 mM Fe-EDTA and 0.5 mM K_2SO_4] with or without 500 μM P_i . Five days after transfer, leaves and roots were harvested and stored at $-70^\circ C$ until further analysis. Rice cell cultures were maintained in the laboratory as described earlier [23]. Phosphorus deficiency treatments were initiated seven days after subculturing the cells. From a cell suspension, cultures growing in 25 mL P_i sufficient media were filtered through Miracloth (Calbiochem, CA) and washed with P_i deficient (0) or sufficient (500 μM) medium. The cells were transferred to the same medium used for washing. The samples were collected at the indicated times after transferring the cells to P_i -deficient medium and then centrifuged at $400 \times g$ for 4 min; the resulting pellet was then used for RNA isolation. The deficiency of the other nutrients was imposed by removing the nutrients (N, K, and Fe) from the solution for three days. *Arabidopsis* plants growth conditions followed the previously described method [24]. Seven-day-old seedlings were transferred to basal medium containing $1 \times$ Murashige and Skoog (HP) micronutrients, $1/5 \times$ macronutrients, 3% sucrose, 2.5 mM MES, B5 vitamins, 1.2% agar or MS LP medium (substitute 0.6 mM K_2SO_4 for 1.2 M KH_2PO_4) for 7 days.

2.2. DNA and RNA Gel Blot Analysis

Total genomic DNA was isolated from rice leaves using the cetyltrimethyl ammonium bromide extraction method [25]. Five micrograms of genomic DNA digested with *EcoRI* and *XhoI* were separated by electrophoresis on 1.2% (w/v) agarose gels and blotted onto nylon transfer membranes (Amersham).

Total RNA was isolated from rice plants and rice cell cultures using TRIzol reagent (Sigma). Total RNA (10 μg) was separated by electrophoresis on 1.2% (w/v) denaturing formaldehyde agarose gels and blotted onto nylon transfer membranes (Amersham).

The membranes were hybridized at $65^\circ C$ for 12 h with a [α - ^{32}P]-dCTP-labeled probe in a solution containing 20% (w/v) SDS, $20 \times$ SSPE, 100 g/L PEG (molecular weight 8000), 250 mg/L heparin, and 10 ml/L herring sperm DNA. The filters were washed twice in $2 \times$ SSC and 0.2% (w/v) SDS at room temperature for 10 min, twice in $1 \times$ SSC and 0.2% (w/v) SDS at $65^\circ C$ for 15 min, and twice in $0.1 \times$ SSC and 0.2% (w/v) SDS at $65^\circ C$ for 20 min before autoradiography.

2.3. Measurement of P_i Concentration

Inorganic P_i measurement followed the previously de-

scribed method [26]. A frozen sample (approximately 0.5 g) was homogenized in 1 mL of 10% (w/v) PCA, using an ice-cold mortar and pestle. The homogenate was then diluted 10 times with 5% (w/v) PCA and placed on ice for 30 min. After centrifugation at 10,000 ×g for 10 min at 4°C, the supernatant was collected to measure the P_i, using the molybdate-blue method: 0.4% (w/v) ammonium molybdate melted in 0.5 M H₂SO₄ (solution A) was mixed with 10% ascorbic acid (solution B) (A:B = 6:1). Two milliliters of this solution was added to 1 mL of the sample solution and incubated in a water bath at 40°C for 20 min. After being cooled on ice, the absorbance was measured at 820 nm.

2.4. Histochemical Localization of GUS Expression

Histochemical staining for GUS activity was performed according to the protocol described by Raghothama *et al.* [27], with some modifications. Whole seedlings or parts of plants to be stained were incubated in GUS reaction mix (25 mg of 5-bromo-4-chloro-3-indolyl-d-glucuronide in 50 mL of 100 mM sodium P_i buffer with 0.1% [v/v] Triton X-100) for 16 h. The stained seedlings were transferred to 70% (v/v) alcohol to remove chlorophyll.

2.5. Preparation of Construct

To amplify the cDNA of *OsMAPK2*, a reverse transcription mix (1 µL) was used in 50 µL PCR reactions containing *OsMAPK2*-specific primers and high-fidelity Taq polymerase LaTaq (TaKaRa). The following PCR conditions were employed: denaturation for 30 s at 94°C, annealing for 30 s at 55°C, and extension for 1 min at 72°C. After 35 cycles of amplification, the PCR product was examined by agarose gel electrophoresis with ethidium bromide staining. A PCR fragment of the expected size was purified from the gel and cloned into the plasmid vector pGemT-easy (Promega). The inserts in selected positive clones were sequenced commercially (Genotech). The *OsMAPK2* gene inserted into pGemT-easy was excised by *Bam*HI restriction digestion and cloned in the sense orientation into the *Bam*HI sites of the pCambia1300-35S vector. The construct was introduced into *Agrobacterium tumefaciens* (EHA105) by electroporation.

2.6. Plant Transformation

Agrobacterium tumefaciens-mediated *Arabidopsis* transformation was performed using the flower vacuum infiltration procedure described by Clough and Bent [28]. Transgenic T1 plants were selected on B5 medium containing carbenicillin (100 mg/L) and hygromycin (20 mg/L) and then transferred to soil and allowed to self-pollinate. Homozygous plants were selected and sub-

jected to growth assays under P_i-depleted conditions. Rice plants were transformed with *A. tumefaciens* as described by Hiei *et al.* [29].

2.7. qRT-PCR Analysis

The total RNA of the rice seedlings was extracted with an RNeasy Plant mini kit (Qiagen). After treatment with DNase to remove genomic DNA contamination, the first strand of cDNA was synthesized by M-MLV reverse transcriptase using 3 µg of total RNA as a template. qRT-PCR was performed with a Bio-Rad CFX system using SYBR with the following reaction conditions: 95°C for 10 min, then 40 cycles of 95°C for 15 s, 60°C for 15 s and 72°C for 15 s. Primers for qRT-PCR analysis are shown in Supplement Table S1, and actin was used as an internal control.

2.8. Statistical Analysis

The results are expressed as the mean values ± SD of at least three replicates are presented. Statistical significance between measurements for different treatments or times was analyzed using Duncan's multiple range test.

3. Results

3.1. Genomic Organization and Expression Patterns of the *OsMAPK2* Gene

To assess the exact copy number of *OsMAPK2* in the rice genome, genomic Southern-blot analysis was carried out using the full-length gene as a probe. We confirmed that wild-type plants contained one copy of the *OsMAPK2* gene in the *O. sativa* genome (Figure 2(a)). We tested whether the *OsMAPK2* gene is induced in rice suspension cell culture at various time intervals. In P_i deficient conditions, the transcript level increases after 30 min and decreased after 1 hour. Northern blot analysis confirmed that in response to N, P, K, and Fe deficiency treatments, the *OsMAPK2* gene was induced very rapidly and strongly (within 1 h after exposure to -N, -P, -K, -Fe nutrient medium). This gene exhibited a stronger increase in transcript abundance in response to P and Fe deficiency (Figure 2(b)), and was induced by P_i deficiency within 30 min (Figure 2(c)). Hydroponic experiments were conducted using Dongjin wild-type rice and culture solution with 500 µM P_i or 0 µM P_i for 5 days. The *OsMAPK2* gene was induced strongly during P_i deficiency in roots and was expressed weakly in shoots (Figure 2(d)).

3.2. Tissue Localization of *OsMAPK2*

To test whether the region upstream of the alternative translation start site of the *OsMAPK2* promoter regulates gene expression, we cloned approximately 1.5 kb up-

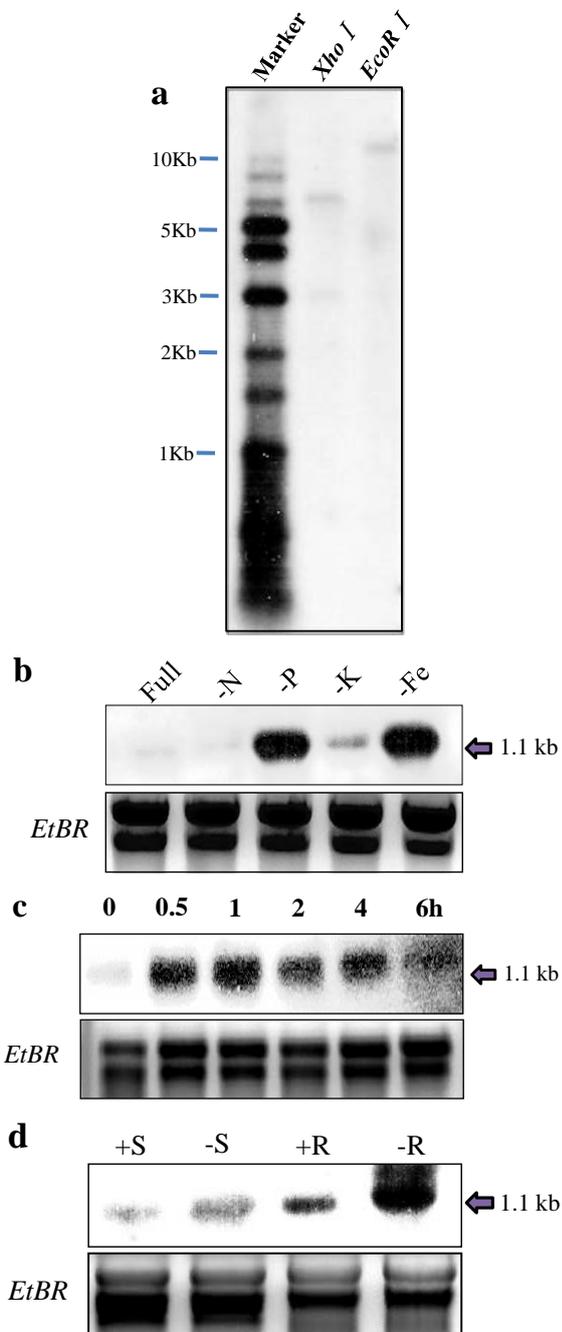


Figure 2. Hybridization analysis of *OsMAPK2* genomic DNA and mRNA. (a) Southern-blot analysis for the wild-type using the *OsMAPK2* gene as a probe. Five micrograms of genomic DNA were digested by *Xho*I and *Eco*RI and separated by agarose gel. Northern blot analysis of the *OsMAPK2* gene; (b) Cell suspension cultures were provided with medium deficient in P_i (-P), nitrogen (-N), potassium (-K), or iron (-Fe), and a control solution with all nutrients (+) for 1 hour; (c) Expression of the *OsMAPK2* gene was analyzed using RNA isolated from a rice suspension cell culture grown in the absence of P_i (-) for the indicated time; (d) Total RNA isolated from the roots and leaves of aerobically grown plants supplied with Hoagland solution containing 500 μ M P_i (+) or no P_i (-) for 5 days.

stream of the translation start sites of the *OsMAPK2* gene and created transgenic lines carrying the GUS gene. Transgenic plants were confirmed for the presence of the appropriate expression cassette by PCR using specific primers (data not shown). Histochemical staining for GUS activity showed that strong GUS expression was observed in the roots of the whole plant 7 days after germination (Figure 3(a)) and in the lateral roots of transgenic plants 21 days after germination (Figure 3(d)). GUS expression was also detected in the mature spikelets in the reproductive stage (Figure 3(b)). Although weak signals appeared for the mature leaves, they were irregular and unstable (Figure 3(c)).

3.3. Alteration of P_i Contents in *OsMAPK2* Transgenic Plants

To investigate the function of this gene, we established transgenic rice plants that overexpressed *OsMAPK2* (*OsMAPK2*-Oe) under the control of the cauliflower mosaic virus 35S constitutive promoter.

Genomic Southern-blot analysis was performed and the transgenic plants contained one copy of a *hygromycin* probe (Figure 4(a)). The expression levels of the transgene were elucidated by Northern-blot analysis (Figure 4(b)) and qRT-PCR (Figure 4(c)). We observed high expression in transgenic plants, but no expression level was obtained from the wild-type plant.

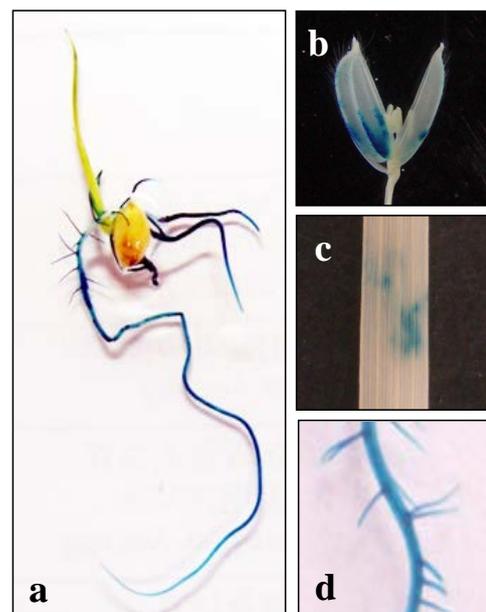


Figure 3. Histochemical analysis of *OsMAPK2*-GUS protein levels in transgenic rice plants. The GUS activity of *OsMAPK2* of the transgenic rice plants at different developmental stages was analyzed. (a) The whole plant 7 days post-germination; (b) Mature spikelets before anthesis at flowering; (c) mature leaves; (d) a 3- to 5 cm section from the root tip at 21 days post-germination.

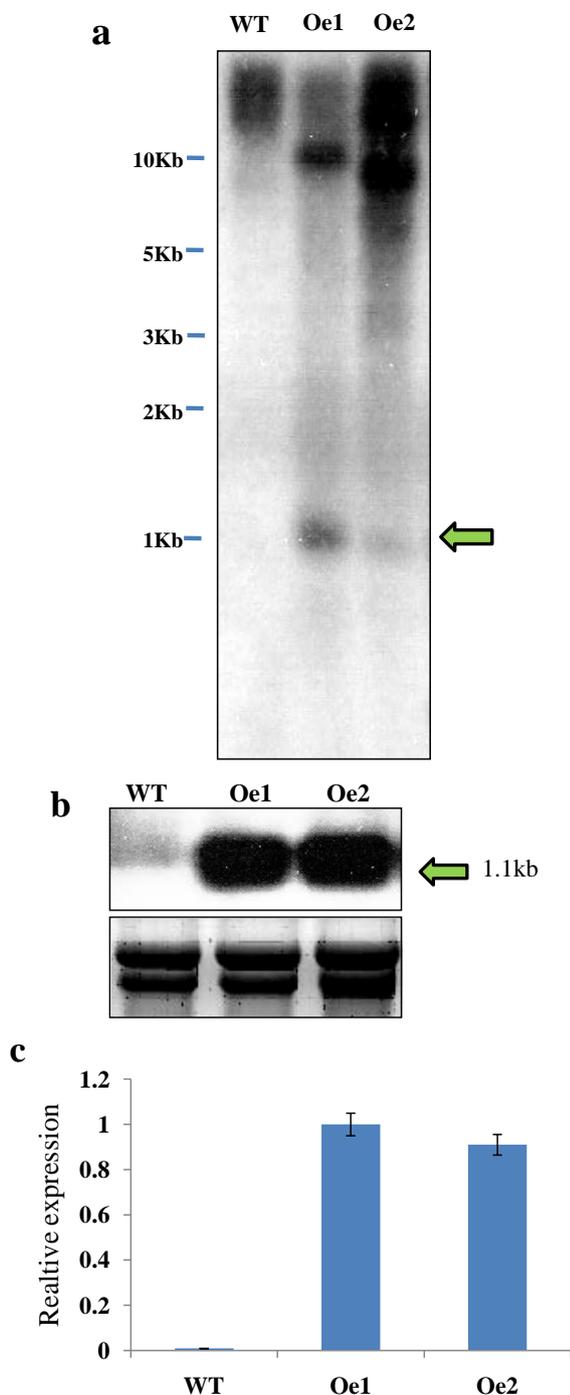


Figure 4. Molecular characterization of *OsMAPK2* overexpression lines. (a) Southern blot analysis of WT and transgenic rice plants. Genomic DNA was restricted from transgenic plants by *XhoI* and then separated on a 1.0% agarose gel. A 1.1-kb full-length DNA fragment of the hygromycin gene was used as the probe; (b) Northern blot analysis for *OsMAPK2* expression level. Total RNA extracted from the shoots of transgenic rice plants was used for analyzing the expression of *OsMAPK2*; (c) qRT-PCR analysis of the expression levels of two transgenic lines with overexpression of *OsMAPK2*. ** and * indicate significant differences relative to the control at $P < 0.01$ and $P < 0.05$, respectively.

To evaluate the potential function of *OsMAPK2*, the growth performance and P_i contents in leaves and roots of the *OsMAPK2* and WT rice plants were investigated in a Hoagland solution culture with P_i sufficient and P_i deficient conditions for 4 weeks (**Figure 5(a)**). The shoot and root fresh weight of the *OsMAPK2*-Oe plants in HP conditions were 6.1% and 9.6% higher than in wild-type

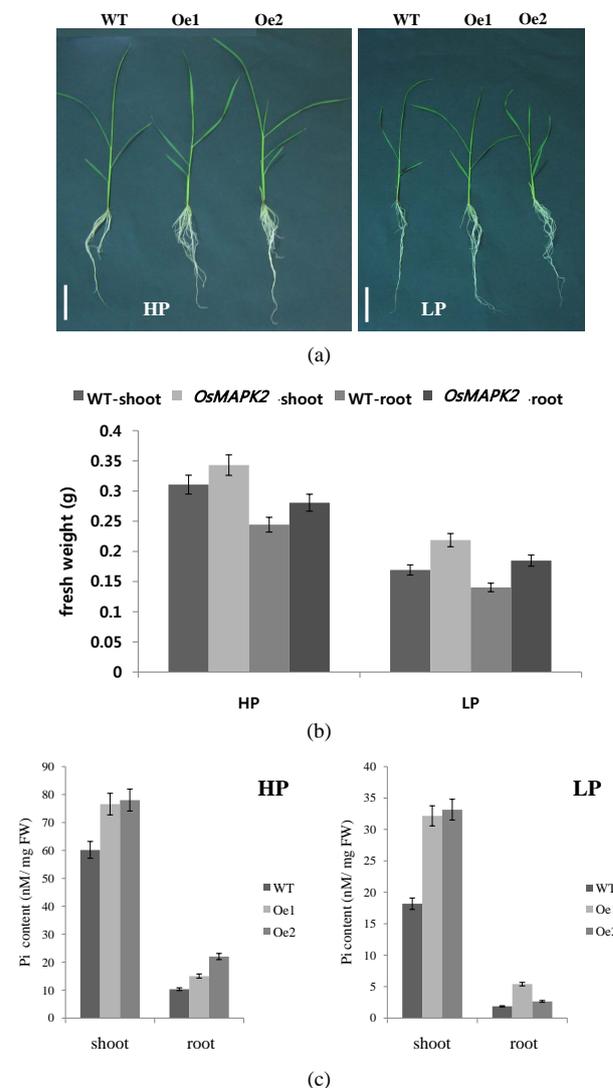


Figure 5. Phenotypic analysis of the *OsMAPK2* transgenic lines and wild-type (WT) rice plants a Growth of 30-day-old seedlings of wild-type and *OsMAPK2* in hydroponic solutions with P_i sufficient (HP) and P_i deficient (LP) conditions for 3 weeks. Scale bars represent 5 cm; (b) Fresh shoot and root weight of WT and *OsMAPK2* transgenic rice plants under HP and LP conditions. Five plants per line were measured. Error bars indicate the SD ($n = 5$); (c) P_i contents in the shoots and roots of wild-type (WT) and *OsMAPK2* (*Oe1*, *Oe2*) plants in hydroponic solutions with P_i sufficient (HP) and P_i deficient (LP) conditions for 3 weeks. Error bars indicate the SD ($n = 4$). ** and * indicate significant differences relative to the control at $P < 0.01$ and $P < 0.05$, respectively.

plants, respectively (Figure 5(b)). In LP conditions, the shoot and root fresh weight of the *OsMAPK2*-Oe plants was approximately 28% higher than that of wild-type plants. The P_i contents from the transgenic lines and wild-type were remarkably different. The P_i contents in shoots of the *OsMAPK2*-Oe plants grown at HP or LP condition were 26% and 66% higher than those in wild-type plants, respectively (Figure 5(c)). These results suggest that there may be a different regulatory mechanism downstream of *OsMAPK2* that controls P_i homeostasis.

3.4. Expression of P_i Starvation-Induced Genes in WT and *OsMAPK2* Transgenic Plants

The expression patterns of well-characterized P_i -starvation-inducible genes (PSI) in rice were examined by qRT-PCR analysis in the roots of 14-day-old *OsMAPK2*-Oe and WT plants grown under both P_i -sufficient and deficient conditions for 7 days (Figure 6). We examined

the expression of *OsIPSI* {a member of the Mt4/TPSI1 family in rice that is induced strongly by phosphate starvation [30]}, *OsSPX1* {a negative factor for the accumulation of P_i in leaves [31]}, *OsPHO2* {the potential ortholog of *AtPHO2*; its mutant is identified as a P_i over-accumulator [32]}, and the phosphate transporters (*OsPT2*, *OsPT4* and *OsPT8*). The expression level of these PSI genes was controlled by overexpression of *OsMAPK2*. While *OsMAPK2*-Oe decreased the expression levels of *OsIPS*, *OsSPX1*, *OsPT2*, *OsPT4* and *OsPT8*, the expression level of *OsPHO2* was increased in P_i deficient condition. It could be that due to the higher internal P_i contents, the response of the PSI genes is weaker in the *OsMAPK2*-Oe plants compared to WT plants.

3.5. Generation of Transgenic Arabidopsis Plants Overexpressing *OsMAPK2*

To determine whether *OsMAPK2* confers similar effects

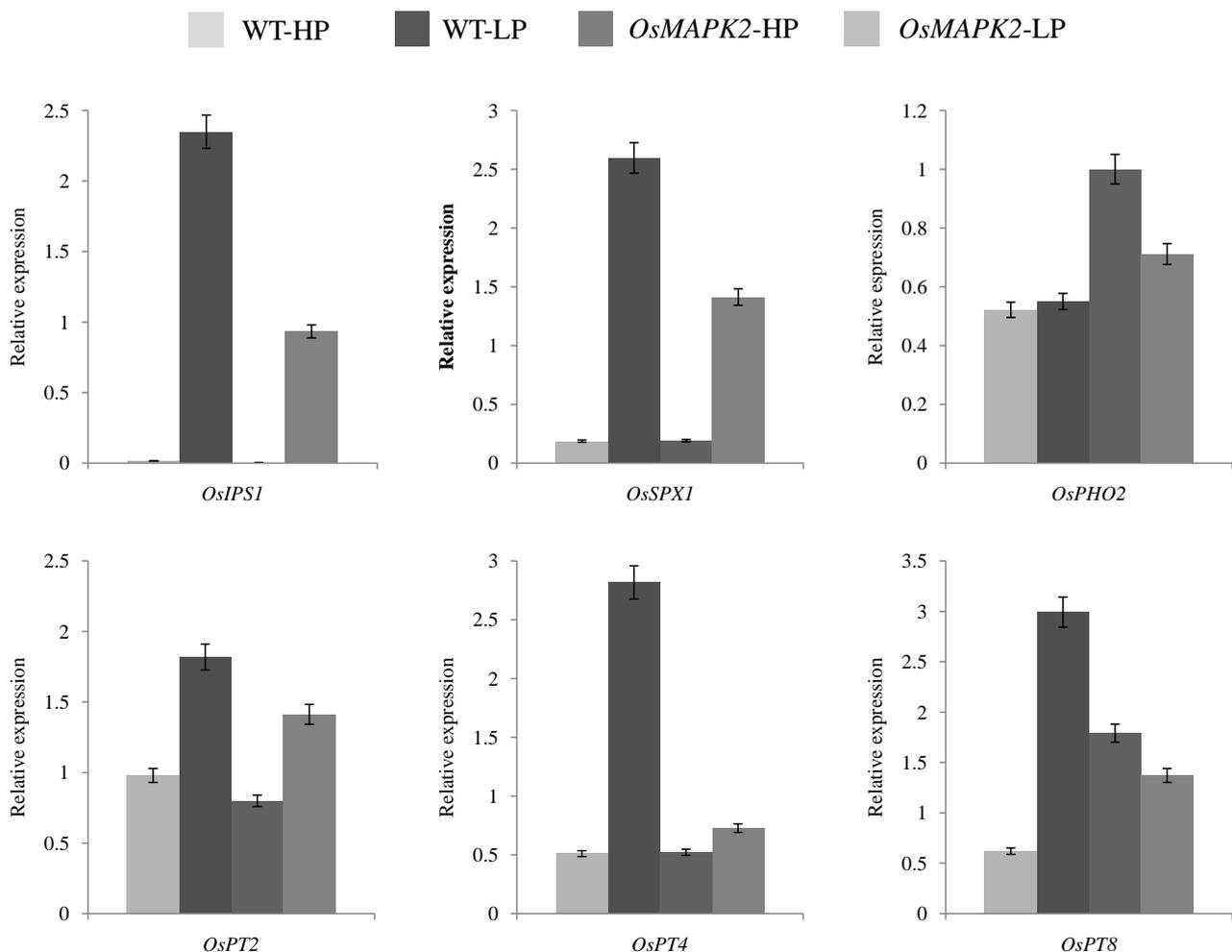


Figure 6. Real-time PCR analysis of P_i starvation-induced genes in the seedlings of wild-type (WT) and *OsMAPK2* rice plants under (A) P_i sufficient (HP) and (B) P_i deficient (LP) conditions for five days. The rice *Actin1* gene was amplified as the internal control. ** and * indicate significant differences relative to the control at $P < 0.01$ and $P < 0.05$, respectively.

in *Arabidopsis*, we transformed *Arabidopsis* with the *OsMAPK2* gene driven by the 35S constitutive promoter. Overexpression of *OsMAPK2* was confirmed by PCR and Northern blot analysis, and both transgenic lines showed significantly enhanced levels of *OsMAPK2* mRNA transcripts compared with control wild-type plants (data not shown).

Seven day-old seedlings of *OsMAPK2*-Oe *Arabidopsis* and WT plants grown in one-half-strength Murashige and Skoog medium were transferred to Murashige and Skoog medium supplemented with high (HP) and low (LP) P_i (1.25 and 0.0125 mM) for 7 days (Figure 7(a)).

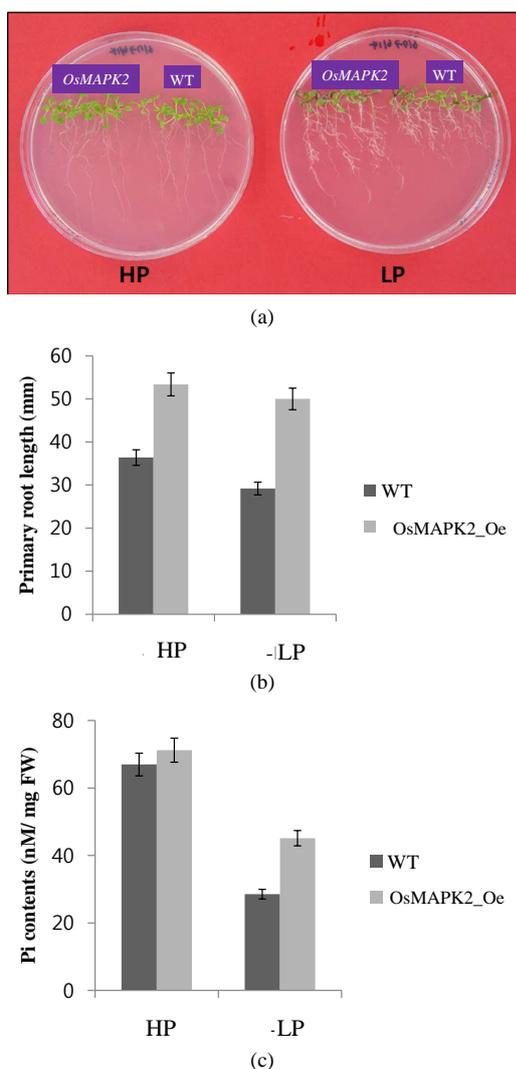


Figure 7. Growth performance of wild-type (WT) and *OsMAPK2* transgenic *Arabidopsis* plants. (a) Phenotype comparison of the WT and transgenic plants. The 7-day-old seedlings germinated on MS medium were transferred to HP or LP medium for another 7 d; (b) Primary root length and (c) P_i contents in the wild type and *OsMAPK2* transgenic plants in HP or LP conditions. ** and * indicate significant differences relative to the control at $P < 0.01$ and $P < 0.05$, respectively.

There was an increase in the primary root length of the *OsMAPK2* plants compared with the wild-type plants, 36% and 72% under HP and LP conditions, respectively (Figure 7(b)). Regarding the P_i contents, the transgenic plants showed slightly increased P_i compared with wild-type plants under HP conditions, and a 42% increased under LP conditions (Figure 7(c)). Our results suggest that *OsMAPK2* also plays a role in regulating the accumulation of P_i in *Arabidopsis* plants.

4. Discussion

MAPK cascades are evolutionarily conserved signaling modules that play an important role in plant responses to multiple stresses. Protein phosphorylation/dephosphorylation modifications are essential in intracellular signaling pathways. Recent studies showed that plant MAPKs are activated by many kinds of abiotic stresses and pathogens [20,33,34].

Earlier studies suggested that *OsMAPK2* could be induced during multiple stresses [19]. In this study, through transgenic analysis, we investigated the role of the *OsMAPK2* gene, which is involved in regulating root development and P_i homeostasis. To the best of our knowledge, *OsMAPK2* is the first member of the MAP kinase genes to be identified as having P_i stress tolerance in plants.

Protein kinase activity has been examined in many studies of kinase [35-37]. Previous study demonstrated that the *OsMAPK2* can phosphorylate itself [38]. *OsMAPK2* was induced within the first 30 min to 1 hour in rice suspension cell culture during P_i deficient conditions and then decreased. In addition, *OsMAPK2* is responsive to both P_i and Fe deficiency. This result showed *OsMAPK2* could also play an important role in the regulation of Fe nutrient deficiency. We have shown that *OsMAPK2* is expressed in both seedling leaves and roots under normal conditions, and higher relative amounts of *OsMAPK2* transcripts were detected in the roots during P_i deficient conditions compared to those in leaves.

First, we generated transgenic rice plants that overexpress *OsMAPK2* under the control of the CaMV 35S promoter. After characterization of the transgenic rice plants, we generated transgenic *Arabidopsis* plants to confirm the function of *OsMAPK2* in dicotyledonous plants. We observed an increase of the P_i content in shoots and roots of both the *OsMAPK2*-Oe rice and *Arabidopsis* plants had increased the fresh weight of transgenic plants compared with wild-type plants. Changes in root architecture increase the ability of a plant to overcome nutrient stresses [39]. As a result of the changes of root architecture, the P_i content of *OsMAPK2*-Oe plants was higher than that of WT plants in both HP and LP conditions. The increasing rate of fresh weight under LP con-

ditions between the transgenic rice plants and WT plants was higher than under HP conditions. And the increasing rates in P_i content in transgenic plants under LP conditions were similar to those under HP conditions. These results indicate that the increased P_i content of transgenic plants is strongly related with the root development.

The expression level of several PSI genes was reduced in the *OsMAPK2*-Oe plants. Previous study of plant phosphate responsive gene (*OsPHR2*) showed that *OsPHR2* overexpressing rice plants exhibited enhanced root elongation and proliferated root hair growth, and some P_i transporters were up-regulated under HP conditions [40]. These results implicate the increase in P_i content in *OsPHR2*-Oe plants. The expression level of PSI genes regulated by *OsMAPK2* could be reduced by the hypothetical intracellular P_i sensing mechanism [41]. A similar effect has also been observed in case of *ZAT6*, which was identified as an *Arabidopsis* transcription factor functioning in the P_i starvation response [42]. *Arabidopsis* plants overexpressing *ZAT6* showed altered root architecture and the expression of several P_i starvation-responsive genes also decreased in *ZAT6* overexpressing plants.

We tested the expression level of 6 phosphate starvation-induced genes. *OsIPS1* has a rapid and specific response to P_i starvation [30]. *AtIPS* negatively affects plant P_i uptake in *Arabidopsis* [43], but the function of *OsIPS1* remains unclear. *OsSPX1* is a negative regulator in both shoots and roots. Suppression of *OsSPX1* increased leaf P_i content and also increased the expression of P_i transporters in roots [44]. Because *OsIPS1* and *OsSPX1* are up-regulated in the roots of the *pho2* mutant [45], it is possible that the expression of *OsIPS1* and *OsSPX1* decreased and the expression of *OsPHO2* increased with the over-expression of *OsMAPK2* compared with wild-type. We also tested the expression of phosphate transporters up- or down-regulation upon over-expression of *OsMAPK2*. Most of the phosphate transporters are induced by LP conditions; the expression levels of *OsPT2*, 4 and 8 were decreased compared with WT under LP conditions. This result indicated that the transgenic plants contain high P_i contents even under LP conditions.

Although many plant MAPK genes localize in both the cytoplasm and the nucleus, *OsMAPK2* has been shown to localize in the chloroplast. The chloroplast is an important organelle that involved in cellular signaling and the phosphorylation network in plants. STN7 and STN8 are well-characterized kinases that are involved in light dependent thylakoid-protein phosphorylation. The loss-of-function mutants showed changes in chloroplast and nuclear gene expression [46,47]. This result suggests that protein phosphorylation in the chloroplast may be associated with the regulation of plastid and nuclear gene

expression [48]. Tissue-specific expression analysis using the promoter-GUS fusion construct showed that the expression of *OsMAPK2* was predominantly localized to roots and reproductive organs, such as the mature spikelet. Plant root parts are likely to be involved in P_i uptake from the soil solution, and root-specific expression of *OsMAPK2* could enhance P_i stress tolerance. The study of rice *OsMAPK2* transgenic plants may provide an idea of why the overexpression of *OsMAPK2* genes in *Arabidopsis* could lead to higher P_i contents and increasing primary root length.

In conclusion, our results suggest that *OsMAPK2* regulates root development and P_i homeostasis. Overexpression of *OsMAPK2* also affected the expression of downstream genes that play an important role in the phosphate signaling pathway in rice. When plants expressing *OsMAPK2* were grown under low phosphate conditions, the root parts could improve absorption of phosphates and enhance low phosphate tolerance.

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Abbreviations

cDNA: complementary DNA

GUS: b-glucuronidase

HP: high phosphate

LP: low phosphate

M-MLV reverse transcriptase: moloney murine leukemia virus reverse transcriptase

mRNA: messenger RNA

Oe: over-expression

PCA: perchloric acid

PCR: polymerase chain reaction

qRT-PCR: quantitative real time polymerase chain reaction

SD: standard deviation

v/v: volume per volume

WT: wild type

w/v: weight per volume

Supplemental Information

Table S1. Primers used in real time PCR analysis.

Gene	Forward sequence (5'-3')	Reverse sequence (5'-3')
<i>OsActin</i>	CAA CAC CCC TGC TAT GTA CG	CAT CAC CAG AGT CCA ACA CAA
<i>OsIPS1</i>	TTG GCA ATT ATT CGG TGG AT	ACC ATT TCA CCA TCC TCT TTT ATG
<i>OsSPX1</i>	CGA CTT CCA TGG CGA GAT	TCC TCT TGT CAT ACT TCT TGA GAA TC
<i>OsPHO2</i>	CGA GAA TTT TGT CAA GGA GCA	TCA CGA GCA TGT CCA ACA A
<i>OsPT2</i>	CAT CGC CTT CTA CAG CCA G	AGC GCG TTC ATG GTG TTC
<i>OsPT4</i>	GGT ACC ATT TCA CGG CGA TC	TGA CGA GGG AGA TGG AGA AG
<i>OsPT8</i>	CCT ACT TGT GTT TGT CTA TGT G	GTG CCA AAT TGC TGG TCT G