

Mitogen-activated protein kinase OsMPK6 negatively regulates rice disease resistance to bacterial pathogens

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Abstract Mitogen-activated protein kinase (MAPK) cascades play important roles in diverse developmental and physiological processes of plants, including pathogen-induced defense responses. Although at least 17 rice MAPKs have been identified and more than half of these MAPK genes have been shown to be pathogen or elicitor responsive, the exact role of most of the MAPKs in host-pathogen interaction is unknown. Here we report that OsMPK6 is an important regulator in rice disease resistance. Suppressing *OsMPK6* or knocking out of *OsMPK6* enhanced rice resistance to different races of *Xanthomonas oryzae* pv. *oryzae*, causing bacterial blight, one of the most devastating diseases of rice worldwide. The resistant plants showed increased expression of a subset of defense-responsive genes functioning in the NH1 (an *Arabidopsis* NPR1 orthologue)-involved defense signal transduction pathway. These results suggest that OsMPK6 functions as a repressor to regulate rice defense responses upon bacterial invasion.

Keywords Bacterial blight · MAP kinase · *Oryza sativa* · Signal transduction

Abbreviations

MAPK Mitogen-activated protein kinase
PR Pathogenesis-related

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RNAi RNA interference
SA Salicylic acid
SAG Salicylic acid β -glucoside
Xoo *Xanthomonas oryzae* pv. *oryzae*

Introduction

Plants respond to a diversity of biotic attackers by regulating the expression of a group of genes that perceive, transduce, and act on defense signals. The mitogen-activated protein kinase (MAPK) cascades are important in various transduction pathways, including pathogen-induced defense signal transduction (Bent 2001; Tena et al. 2001; Asai et al. 2002). Each MAPK cascade consists of at least three evolutionarily conserved signaling molecules, MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK; MAPKKK activates MAPKK, which in turn activates MAPK through phosphorylation. Within the signaling pathway(s), MAPK phosphorylates its downstream component(s), such as transcription factor(s), leading to the activation or suppression of the function of the phosphorylated component(s) (Tena et al. 2001; Asai et al. 2002). Members of MAPK cascades belong to large families, for instance, there are 20 MAPK genes, 10 MAPKK genes, and more than 80 MAPKKK genes in the *Arabidopsis* genome (Ichimura et al. 2002; Hamel et al. 2006). The same MAPK pathway may transduce different signals, and the same signal or physiological process may be regulated by different MAPK pathways or cross-linked MAPK pathways (Cardinale et al. 2000; Nuhse et al. 2000; Tena et al. 2001; Asai et al. 2002; Jonak et al. 2002).

MAPKs are the central components that link MAPK cascades and downstream targets. Several MAPKs from different plant species have been demonstrated to play a role in

pathogen-induced defense signal transduction by function-complementary experiments. A few other MAPKs have been proposed to be involved in the regulation of defense responses because their expression is influenced by pathogens or pathogen elicitors. NtSIPK and NtWIPK, two early discovered MAPKs from tobacco, are involved in pathogen-induced defense responses (Zhang and Klessig 1998; Romeis et al. 1999). The two MAPKs are activated by general and race-specific pathogens (Pedley and Martin 2005). *Arabidopsis* AtMPK3 and AtMPK6 are homologues of tobacco NtWIPK and NtSIPK, respectively, and activation of these two MAPKs confers resistance to both bacterial and fungal pathogens (Zhang and Klessig 2001; Asai et al. 2002). Another *Arabidopsis* MAPK, AtMPK4, was identified as a negative regulator of systemic acquired resistance, but it was required for jasmonic acid-responsive gene expression (Petersen et al. 2000). NtMPK4 is a tobacco homologue of AtMPK4 (Gomi et al. 2005); it is also involved in jasmonic acid signaling, although its role in disease resistance is unknown. A cucumber MAPK, CsTIPK, functions as an activator in disease resistance (Shoresh et al. 2006). Three parsley MAPKs, PcMPK3, PcMPK4, and PcMPK6 that are the homologues of AtMPK3, AtMPK4, and AtMPK6, respectively, are activated by pathogen elicitors, suggesting their putative involvement in disease resistance (Kroj et al. 2003). Four alfalfa MAPKs, MsSAMK (also known as MsMMK4; Tena et al. 2001), MsSIMK, MsMMK2, and MsMMK3, are induced by fungal elicitors, suggesting their possible involvement in pathogen-host interaction (Cardinale et al. 2000). The tomato MAPKs, LeMPK2 and LeMPK3, are activated in Pto-mediated disease resistance (Pedley and Martin 2004). The potato MAPK StMPK1 is elicitor responsive and may be involved in regulation of plant cell death (Katou et al. 2005).

Although 17 rice MAPKs have been identified (Reyna and Yang 2006), little is known about their function. Among these rice MAPKs, only two were confirmed to be involved in pathogen-induced defense regulation based on function-complementary experiments. The first cloned rice MAPK, BWMK1 (also known as OsMPK12; Reyna and Yang 2006), was induced upon blast pathogen infection (He et al. 1999). A recent study revealed that BWMK1 phosphorylates transcription factor(s) that in turn regulate defense gene expression; overexpression of BWMK1 in tobacco enhanced resistance to pathogens (Cheong et al. 2003). OsMAPK5 (also known as OsMAP1, OsMAPK2, OsMSRMK2, and OsBIMK1) is a negative regulator of disease resistance, and its suppression can enhance rice resistance to both fungal and bacterial pathogens (Agrawal et al. 2002; Song and Goodman 2002; Xiong and Yang 2003). An additional eight rice MAPK genes, *OsMPK1* (also known as *OsMAPK6*), *OsMPK2*, *OsMPK4*, *OsMPK7*, *OsMPK8*, *OsMPK13*, *OsMPK15*, and *OsMPK17*, were

induced by rice fungal elicitors, suggesting that they may play roles in defense signal transduction (Lieberherr et al. 2005; Reyna and Yang 2006).

In this study we characterized and performed functional analysis of the *OsMPK6* gene according to the rice MAPK numbering system of Reyna and Yang (2006). *OsMPK6* is the homologue of *Arabidopsis* AtMPK4 and tobacco NtMPK4. Function-complementary experiments showed that *OsMPK6* negatively regulates defense signal transduction in resistance to bacterial blight, the most devastating bacterial disease of rice in the world.

Materials and methods

Determination of *OsMPK6* structure

The sequence of cDNA clone EI106L06 (Zhang et al. 2005) of the *OsMPK6* gene from rice variety Minghui 63 (*Oryza sativa* ssp. *indica*) was used to screen the GenBank nucleotide sequence database for homologous genomic sequence using the BLAST program (Altschul et al. 1997). The rice genomic sequence identified by a BLAST search was analyzed using the GenScan program (Burge and Karlin 1997) to predict the size and structure of the gene homologous to the cDNA sequence EI106L06. The analysis indicated that EI106L06 was the full-length cDNA of *OsMPK6*. The homologous genomic sequence of EI106L06 and its flanking sequences identified by BLAST analysis were used to design overlapping PCR primers. The overlapping PCR fragments of *OsMPK6* were amplified from Minghui 63 using primers MPK1F (5'-ATGAATTCCCA TGGATTCTCCTCCGGC-3'), MPK2F (5'-ATGAATTC TGTACCAGTTGCTACGAGGG-3'), MPKR (5'-ATAAG CTTCTCTGAGCTCTTAGTAGGGAGG-3'), MPK3F (5'-TCTTCATCACCCATACTTG-3'), and MPK3R (5'-TGT CCATGCCTCTACATT-3') and were sequenced.

DNA gel blot and RNA expression analyses

Rice total DNA was digested with *Bam*HI, *Dra*I, *Eco*RV, *Hind*III, or *Pst*I, separated by electrophoresis on 0.8% agarose gels, and blotted onto nylon membranes. A 782-bp probe of the *OsMPK6* gene, amplified using primers MPK2F (5'-ATGAATTCTGTACCAGTTGCTACGAG GG-3') and MPKR (5'-ATAAGCTTCTCTGAGCTCTT AGTAGGGAGG-3'), was used for hybridization.

RNA was extracted and 20 µg of total RNA was used for RNA gel blot analysis as described previously (Chu et al. 2004) using the same 782-bp probe as for DNA gel blot analysis. The other hybridization probes were cDNA clones BI145L12 (GenBank accession no. CX099614), EI095E11 (CX101294), EI060B22 (CX111338), BI122B17

(CX099537), EI089H13 (CX109097), and BI147O12 (CX099696) from the Minghui 63 cDNA library (Zhang et al. 2005) for *PR5* (X68197, thaumatin-like protein), *PR10* (or *PBZ1*, D38170, ribonuclease), *WRKY03* (AY 676924), *NHI* (AY923983, Arabidopsis NPR1 homologue 1), *PAL* (X16099, phenylalanine ammonia-lyase), and *Chl1* (D16221, chitinase 1), respectively.

Rice transformation

To construct an RNA interference (RNAi) vector of *OsMPK6*, a 755-bp cDNA fragment of *OsMPK6* amplified using primers dsF (5'-TAACTAGTGGCGCCTGCAGG TACCGGTCCG-3') and dsR (5'-TAGAGCTCGCCTAGG TGCACGCGTACGTACGTAAGC-3') from the truncated cDNA clone EI106L06, which was obtained by digesting clone EI106L06 with *NotI* and *AccI* followed by self-ligation, was inserted into the pDS1301 vector (Supplemental Fig. 1a; Chu et al. 2006). The dsF contained *SpeI* and *AscI* restriction enzyme sites (underlined) at the 5'-end, and dsR contained *SacI* and *AvrII* restriction enzyme sites (underlined) at the 5'-end. The sequences following the restriction enzyme sites of dsF and dsR were complementary to the sequences flanking the multicloning sites of pSPORT1 vector for cDNA clone EI106L06. RNAi constructs were transferred into *Agrobacterium tumefaciens* strain EHA105 by electroporation. *Agrobacterium*-mediated transformation was performed using calli derived from mature embryos of rice variety Zhonghua 11 (*O. sativa* ssp. *japonica*) (Lin and Zhang 2005).

Pathogen inoculation and histochemical staining

Plants were inoculated with Chinese *Xoo* strain Z174 (group 4) or Philippine *Xoo* strains PXO61 (race 1), PXO71 (race 4), PXO99 (race 6), or PXO145 (race 7) at the 5- to 6-leaf or booting stage by leaf-clipping method (Zhou et al. 2002). Mock-inoculated (control) plants were treated under the same condition except that bacterial inoculum was replaced with water. The bacterial inoculum was prepared as described previously by Zhou et al. (2002). Disease was scored by measuring the percent lesion area (lesion length/leaf length) at 21 days after inoculation. Trypan blue staining of rice leaves was performed using the methods described previously (Yin et al. 2000).

Quantification of salicylic acid

Samples of salicylic acid (SA) were extracted from plant leaves at the booting stage with the procedure described previously (Qiu et al. 2007). In brief, the leaf samples were ground under liquid nitrogen. Three replicates of each ground sample weighing 300–1,000 mg (exact weights were

recorded) were placed in 3 ml of 80% methanol supplemented with 1 µg naphthaleneacetic acid standard as an internal control and left at 4°C for 24 h in the dark. The average recovery rate of this procedure was approximately 70%. The organic extracts containing free SA were analyzed by HPLC (Agilent 1100; Agilent Technologies, Palo Alto, California, USA) with an Agilent C18 Zorbax ODS column (250 × 5 mm) run at 25°C in 50% water (0.1% H₃PO₄)/50% methanol with a flow rate of 0.6 ml/min. SA was detected using a 230-nm wavelength. The aqueous phase containing SA conjugate (SA β-glucoside, SAG) was acidified with HCl to pH 1 and boiled for 30 min to release SA from any acid-labile conjugated forms. The released free SA was then extracted with the organic mixture and analyzed.

Results

OsMPK6 gene

Comparative analysis of the genomic sequence of *OsMPK6* (GenBank accession no EF174189) from rice variety Minghui 63 and its full-length cDNA sequence showed that this gene was 3,735 bp in length and consisted of seven exons encoding a protein of 376 amino acids (Supplemental Fig. 1b). The *OsMPK6* gene showed 99% sequence identity with its alleles in *japonica* rice variety Nipponbare (AC092389) and another *indica* variety 93-11 (AAAA02030404). The encoding product of *OsMPK6* in Minghui 63 was identical to that of the corresponding alleles in Nipponbare and 93-11. DNA gel blot analysis showed that both *japonica* and *indica* rice genomes contained only one copy of an *OsMPK6*-homologous gene (Supplemental Fig. 2). When compared with the pathogen-responsive MAPKs of other plant species, *OsMPK6* showed the highest sequence similarity with *Arabidopsis* AtMPK4 (National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>) protein database accession no AAZ20637, 84% sequence identity and 92% sequence similarity).

OsMPK6 suppression enhances rice resistance to bacterial blight

The RNAi technique was used to suppress *OsMPK6* expression in rice variety Zhonghua 11. Thirty-six independent transformants named D87RZ1-1 to -36 were obtained. These T₀ plants showed no obvious phenotypic changes during the early developmental stage. However, twelve plants developed dark brown lesion mimics (i.e., spots of dead cells that resemble infection) on the leaves at the booting stage in the field (Fig. 1a). Staining the leaves with visible lesion mimic using trypan blue, an indicator of dead cells (Yin et al. 2000), showed dark blue stained spots at

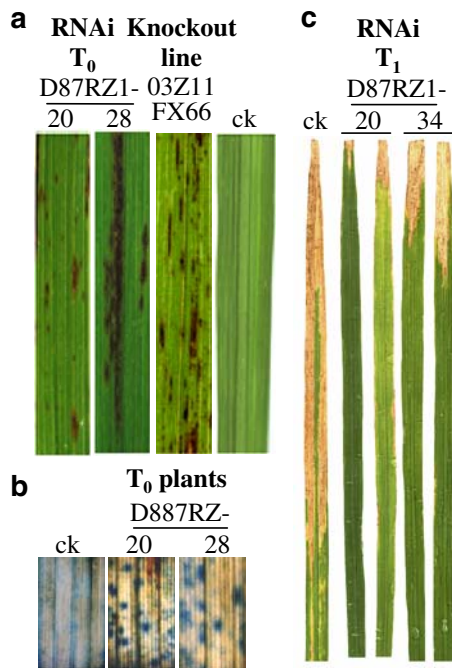


Fig. 1 Phenotype of transgenic plants. Zhonghua 11 is wild type (*ck*). **a** *OsMPK6*-suppressing (D87RZ1) and *OsMPK6*-knockout (03Z11FX66) plants developed lesion mimics on their leaves at booting stage. **b** Trypan blue staining of leaves with visible lesion mimics. **c** *OsMPK6*-suppressing plants showed enhanced resistance to *Xoo* strain PXO61 compared to the wild type at 21 days after inoculation

the sites of some lesion mimics and sites adjacent to lesion mimics (Fig. 1b); such dark blue spots was not observed in the leaves of wild type. The wild type was susceptible to *Xoo* strain PXO61. All the T_0 transgenic plants were inoculated with PXO61 at the booting stage. Twelve of the 36 plants, which developed lesion mimic, showed significantly enhanced resistance, with the lesion area ranging from 5 to 37%, as compared to 71% measured for the wild type (Fig. 2a). Bacterial growth analysis demonstrated that the growth rate of PXO61 on resistant T_0 transgenic plants was 7–72-fold lower than that on wild type at 4–16 days after inoculation (Fig. 3). The transgenic plants that showed no significant difference ($P > 0.05$) in the size of lesion area compared to the wild type did not develop lesion mimics. The reduced lesion area of the T_0 plants was highly correlated with reduced *OsMPK6*-transcript level (Fig. 2a; $r = 0.849$, $\alpha = 0.01$, $n = 14$).

Two T_1 families from resistant T_0 plants, D87RZ1-20 and D87RZ1-34, were further analyzed for their resistance to *Xoo* as well as *OsMPK6*-transcript level (Fig. 1c). The enhanced resistance cosegregated with the reduced *OsMPK6* transcripts in D87RZ1-20 T_1 plants (Fig. 2b); the correlation between lesion area and *OsMPK6* expression level in this family was 0.793, significant at $\alpha = 0.01$ ($n = 16$). Similar result was also observed for D87RZ1-34 family ($r = 0.898$, $\alpha = 0.01$, $n = 21$). The *OsMPK6*-sup-

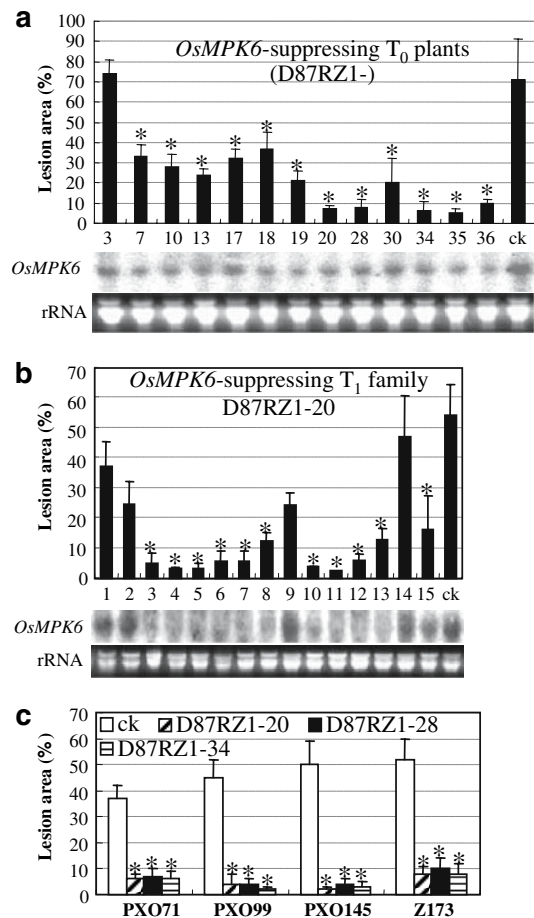


Fig. 2 Resistance of *OsMPK6*-suppressing plants to *Xoo*. The asterisk indicates that a significant difference ($P < 0.01$) in lesion area was detected between transgenic (D87RZ1) and wild-type (Zhonghua 11, *ck*) plants. Bars represent mean \pm standard deviation. The quantitative data of RNA gel blot analyses in **a**, **b** are shown in Supplemental Figs. 3a, b, respectively. **a** The resistance of transgenic plants to *Xoo* strain PXO61 was associated with reduced expression of *OsMPK6*, as detected by RNA gel blot analysis. D87RZ1-3 is a negative transgenic plant, and others are positive transgenic plants. **b** The enhanced resistance cosegregated with suppression of *OsMPK6* in the T_1 family. **c** The transgenic plants (T_1) were also resistant to *Xoo* strains PXO71, PXO99, PXO145 and Z173

pressing plants began to develop lesion mimics at the booting stage. Only a few resistant T_1 plants developed visible lesion mimics before pathogen inoculation at the booting stage, and the rest of the resistant T_1 plants developed lesion mimics after pathogen inoculation. Three homozygote *OsMPK6*-suppressing lines (D87RZ1-20, -28 and -34) were also inoculated with PXO61 at 5- to 6-leaf stage, which were free of lesion mimic. All the transgenic plants were resistance to *Xoo* (Supplemental Fig. 4). These results suggest that *OsMPK6* may function as a negative regulator in the pathogen-induced defense response: suppression of *OsMPK6* expression can cause spontaneous, stage-dependent lesion mimics, but enhanced resistance does not require the development of lesion mimic.

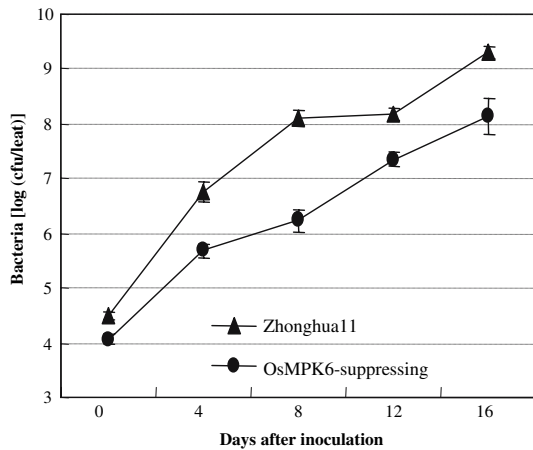


Fig. 3 Growth of *Xoo* strain PXO61 in leaves of *OsMPK6*-suppressing plants. T₀ plants D87RZ1-20, D87RZ1-28, and D87RZ1-34 regenerated from their stubs after seed harvesting were used for the analysis. Each time point was the average of three lines (mean ± standard deviation). Zhonghua 11 is the wild-type plant. The bacterial population was determined from three leaves at each time point by counting colony-forming units (cf. Qiu et al. 2007)

Plants from three independent T₁ families were further examined for their resistant spectrum to *Xoo*. Pathogen inoculation analysis showed that these *OsMPK6*-suppressing plants were also resistant to *Xoo* strains PXO71, PXO99, PXO145 and Z173, which represent different *Xoo* races (Fig. 2c). The lesion area on the transgenic plants caused by the invasion of bacteria was 81–96% smaller than that of the wild type. These results suggest that *OsMPK6* suppression can also induce a broad spectrum of resistance to *Xoo*.

A mutant 03Z11FX66 with T-DNA inserted in the intron of *OsMPK6* (Supplemental Fig. 1b) was identified from the Rice Mutant Database (Zhang et al. 2006). The 03Z11FX66 had the genetic background of Zhonghua 11. Some of the 03Z11FX66 plants showed significantly enhanced resistance to PXO61 and this enhanced resistance was associated with the insertion of T-DNA (Supplemental Table 1), suggesting that the enhanced resistance was due to knockout of *OsMPK6*. All the *OsMPK6*-knockout plants developed dark brown lesion mimic at booting stage (Fig. 1a, Supplemental Table 1). The average lesion area of the *OsMPK6*-knockout plants after PXO61 infection was 3 ± 1.6%, as compared to 42 ± 10.1% measured for wild-type plants.

Modulating *OsMPK6* expression influences the transcript levels of a subset of defense-related genes and the level of endogenous salicylic acid

Knockout of *OsMPK6* induced the expression of some known defense-related genes, including pathogenesis-related (PR) genes *PR5* and *PR10/PBZ1*, *PAL*, *NHI*, and *WRKY03*, and showed no influence on the expression of *Chl1* either with or without bacterial challenge (Fig. 4a).

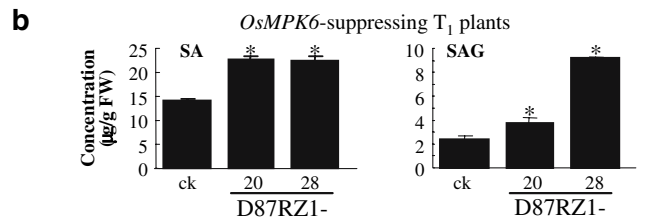
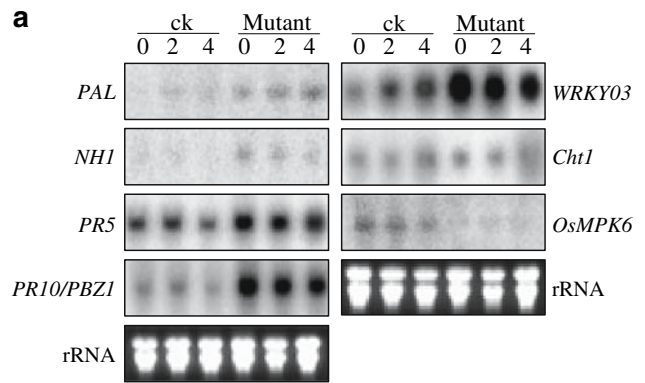


Fig. 4 Knockout/suppression of *OsMPK6* influenced the expression of other defense-related genes (*PAL*, *NHI*, *PR5*, *PR10/PBZ1*, *WRKY03*, and *Chl1*) and the accumulation of salicylic acid (SA) and conjugate SA (SA β-glucoside, SAG). Zhonghua 11 is wild type (ck). **a** Expression of defense-related genes in *OsMPK6*-knockout plants analyzed by RNA gel blot. Samples were collected from *OsMPK6*-knockout mutant 03Z11FX66 before pathogen (PXO61) inoculation (0 day) and at 2 and 4 days after pathogen inoculation (lesion mimics developed). **b** Free SA and SAG levels were measured in the leaves of Zhonghua 11 and *OsMPK6*-suppressing plants (D87RZ1-20, D87RZ1-28). For the transgenic plants, each sample was a mixture from three T₁ plants that had developed lesion mimics. Asterisks indicate a significant difference ($P < 0.05$) between transgenic and wild-type plants. FW, fresh weight. Bars represent mean ± standard deviation

Three *OsMPK6*-suppressing resistant T₀ plants, D87RZ1-20, D87RZ1-28 and D87RZ1-34, were also examined for the expression of the six defense-related genes. The six defense-related genes in *OsMPK6*-suppressing plants showed the similar expression patterns as those in *OsMPK6*-knockout mutant compared to the wild type (Supplemental Fig. 5).

The leaves of *OsMPK6*-suppressing T₁ plants were analyzed for the endogenous levels of SA and SA conjugate. These bacterial resistance plants accumulated 1.6-fold more free SA and 1.5- to 3.8-fold more SAG than the wild-type Zhonghua 11 at booting stage (Fig. 4b).

Discussion

MAPKs belong to a large family of signal proteins, and a relatively large number of MAPKs have been shown to be responsive to pathogen or elicitor challenges, indicating their putative involvement in defense signal transduction. Yet, only a few MAPKs have been characterized with

regard to their exact roles in plant disease resistance. Our molecular and functional characterization of OsMPK6 revealed that it functions as a negative regulator of rice disease resistance. This study provides another example for comparative analysis of the functions of MAPKs in response to pathogen invasion in different plant species.

OsMPK6 regulates defense response via a NH1-involved pathway

The existence of this pathway is strongly supported by the evidence that both *OsMPK6*-knockout and -suppressing plants showed the same phenotype and similar expression patterns of defense-related genes. In this pathway, OsMPK6 functions as a negative regulator to suppress the function of a subset of defense-related genes. Suppression of *OsMPK6* resulted in the accumulation of transcripts of the defense-related genes *WRKY03*, *NH1*, *PAL*, *PR5*, and *PR10*. *WRKY03* is a pathogen-activated transcription activator located upstream of *NH1* and *PAL* (Liu et al. 2005). *NH1* is a rice orthologue of *Arabidopsis* *NPR1* (Chern et al. 2005; Yuan et al. 2007). *NPR1* is a key regulator of systemic acquired resistance (Durrant and Dong 2004). It functions downstream of SA by binding to TGA family members of basic-region leucine zipper transcription factors, which in turn regulate the expression of SA-responsive genes. Overexpression of *NPR1* or *NH1* can enhance rice resistance to *Xoo*; in addition, *NPR1* and *NH1* interact with rice TGA proteins and *NH1* complements the *Arabidopsis* *npr1-1* mutant, suggesting that rice and *Arabidopsis* share a conserved signal transduction pathway controlling *NPR1*-mediated resistance (Chern et al. 2001, 2005; Yuan et al. 2007). Overexpression of *NH1* results in expressional activation of *PAL* and *PR10* (Chern et al. 2005). *PAL* catalyzes the first reaction in the biosynthesis of SA in the phenylpropanoid pathway (Lee et al. 1995). *OsMPK6*-suppressing plants accumulated SA (Fig. 4b). Our analysis also showed that SA induces the expression of *PR10* (D. Qiu et al. unpublished). These results suggest that *PAL* may function upstream of *PR10*. Thus, the successive downstream components of OsMPK6 in this defense signal transduction pathway may be *WRKY03*, *NH1*, *PAL* and *PR10*. However, *PR5*-involved defense signal transduction pathway has not been reported. Further study is needed to determine the position of *PR5* in OsMPK6-regulated signal transduction pathway.

Overexpression of *OsMPK6* in rice variety Zhonghua 11 resulted in two phenotypes of transgenic plants in response to pathogen challenge (data not shown). Most of the *OsMPK6*-overexpressing plants showed no distinct difference from wild type in response to *Xoo* infection, whereas a small number of *OsMPK6*-overexpressing plants showed enhanced *Xoo* resistance when they developed lesion

mimics on the leaves. Possible explanation for the phenotype in resistant *OsMPK6*-overexpressing plants may be that *OsMPK6* overexpression nonspecifically perturbed signal transduction, leading to the development of lesion mimic and enhanced resistance. Overexpression of signaling elements is known to sometimes disturb signal transduction. Two phenotypes have also observed in transgenic tobacco overexpressing MAPK *NtWIPK* (Seo et al. 1999). Induced expression of another tobacco MAPK *Ntf4* resulted in hypersensitive response-like cell death, whereas constitutive expression of *Ntf4* failed to present this phenotype (Ren et al. 2006). Thus, we propose that the negative role of OsMPK6 in defense signal transduction is its basal function in the regulation of rice disease resistance.

Among the characterized plant MAPKs involved in regulation of pathogen-induced defense, only rice OsMAPK5 and *Arabidopsis* *AtMPK4* function as negative regulator and others function as positive regulator. OsMPK6 is the homologue of *AtMPK4* that is a negative regulator of systemic acquired resistance; however, *AtMPK4* did not influence the function of *NPR1*, the key regulator of systemic acquired resistance (Petersen et al. 2000). The pathway of OsMAPK5-involved defense regulation is not known (Xiong and Yang 2003). OsMPK6 regulates rice disease resistance via a *NH1/NPR1*-involved pathway, although it also functions as a negative regulator. The function of two tobacco MAPKs, *NtSIPK* and *NtWIPK*, and their *Arabidopsis* homologues *AtMPK3* and *AtMPK6*, respectively in pathogen-induced defense responses have been extensively studied (Zhang and Klessig 2001; Asai et al. 2002; Pedley and Martin 2005), but none of these defense activators has been reported to directly or indirectly regulate *NPR1*- or its orthologue-involved pathway in disease resistance. Thus the present results provide another view for our understanding of the functions of MAPK in disease resistance.

The lesion mimic may be associated with accumulation of SA

Lesion mimics are spots of dead cells on leaf tissue that resemble infection; these mimics can occur spontaneously but usually develop after abiotic or biotic stress. In the present study, the lesion mimic phenotype of *OsMPK6*-knockout/suppressing plants was not required for enhanced resistance. In a previous study, rice plants overexpressing *NH1* showed the lesion mimic phenotype; the development of lesion mimics in *NH1*-overexpressing plants was not associated with the enhanced resistance but was related to plant age (booting stage) and due, in part, to the high endogenous SA level (Chern et al. 2005). Overexpression of *Arabidopsis* *NPR1* in rice also leads to a lesion mimic phenotype (Fitzgerald et al. 2004). SA is involved in leaf senescence in *Arabidopsis* (Morris et al. 2000). Because the

OsMPK6-suppressing plants showed accumulation of SA, the development of lesion mimics in these transgenic plants may be associated with high endogenous level of SA.

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