식물생명공학회지 제36권 제1호
J Plant Biotechnol
Vol. 36, No. 1, 45-52 (2009)

Identification and characterization of a rice blast fungal elicitor-inducible *Oshin1* gene

Cha Young Kim[1] · Sung-Ho Lee[2*]

[1]*Bioindustry Research Center, Korea Research Institute of Bioscience and Biotechnology, Jeongup 580-185, Korea*

[2]*Department of Biology, Division of Applied Life Science (BK21), PMBBRC and Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, Korea*

ABSTRACT In order to understand the molecular interactions that occur between rice and the rice blast fungus during infection, we previously identified a number of rice blast fungal elicitor-responsive genes from rice (*Oryza sativa* cv. Milyang 117). Here, we report the cloning and characterization of the rice fungal elicitor-inducible gene *Oshin1* (GenBank Accession Number AF039532). Sequence analysis revealed that the *Oshin1* cDNA is 1067 bp long and contains an open reading frame encoding 205 amino acid residues. The *Oshin1* gene shows considerable sequence similarity to the tobacco *hin1* and *hin2* genes. The predicted *Oshin1* protein has a cysteine-rich domain at the N-terminus and is rich in leucine, serine, and alanine residues. Southern blot analysis suggests that *Oshin1* gene is a member of a small gene family in the rice genome. To examine the expression of *OsHin1*, Northern blot analysis was conducted. Expression of the *Oshin1* transcript is rapidly induced in suspension-cultured rice cells treated with fungal elicitor, salicylic acid or hydrogen peroxide. In addition, *Oshin1* transcript levels are rapidly increased by treatment with Ca^{2+}/A23187. The expression of *Oshin1* was also elevated in 3-week old leaf tissues upon ethephon application or fungal elicitor treatment. Our results suggest that the *Oshin1* gene is involved in plant defense responses to environmental stresses.

Introduction

Plants respond to pathogen attack by rapidly activating a variety of defense reactions. These responses are frequently associated with the rapid, localized collapse of infected tissue, a phenomenon known as the hypersensitive response (HR). Localized cell death during HR may restrict the spread of the pathogen from the infection site. The molecular basis for HR is still lacking, but physiological and genetic studies with bacteria suggest that the same factor that triggers HR in non-host plants is also required for pathogenicity in host plants. Production of this unknown factor is controlled by a cluster of highly conserved genes called *hrp* (hypersensitive response and pathogenicity) genes (Wei et al. 1992; Willis et al. 1991). These defense responses sense signals released from pathogens and plants during infection and trigger subsequent signal transduction through intracellular signaling cascades. The elicitor signals include oligosaccharides, proteins, and glycoproteins that are released from the invading pathogens, as well as the plant cell wall (Dixon and Lamb 1990; Ryan and Farmer 1991). Harpin is a proteinaceous elicitor (44 kD) of the plant defense response isolated from the bacterium *Erwinia amylovora*, which causes the fire blight disease of apple, pear, and rosaceous plants. The harpin protein elicits HR in leaves of tobacco, tomato, and *Arabidopsis thaliana* (Wei et al. 1992). Sequences homologous to harpin-induced (hin) genes have been identified in various plants including tobacco, Arabidopsis, potato, rice, pepper, and soybean. Several *hin* genes were identified from tobacco by subtractive hybridization. Among these genes, two classes of *hin* genes (*hin1* and *hin2*) were induced by bacteria and harpin (Gopalan et al. 1996). Interestingly, the induction of *hin1* by bacteria was dependent on functional bacterial *hrp* genes. The *hin1* gene encodes a novel protein of 221 amino acids. The tobacco *hin1* gene is induced during leaf senescence (Pontier et al. 1999), and the Arabidopsis *hin1* homolog (named YLS9, At2g35980) is also expressed at high levels in senescent leaves (Yoshida et al. 2001). We previously identified a large number of rice blast fungal-responsive genes from a rice cell

*Corresponding author Tel 82-55-751-5949 Fax 82-55-754-0086
E-mail: leesh@gnu.ac.kr

culture using an mRNA differential display technique (Kim et al. 2000). Here, we further studied the rice blast fungal elicitor-responsive gene *Oshin1* which is induced by fungal pathogens, salicylic acid (SA), hydrogen peroxide (H_2O_2), 2-chloroethylphosponic acid (ethephon), and calcium treatment. These results suggest that *Oshin1* participates in plant defense responses and may serve as a valuable molecular probe.

Materials and Methods

Plant materials and culture of rice suspension cells

Rice seeds (*Oryza sativa* cv. Milyang 117) were obtained from the Gyeongsang Provincial Development Administration, Korea. Mature seeds were surface sterilized in 70% (v/v) ethanol for 5 min and in a 2% (v/v) solution of sodium hypochlorite for 30 min, and then rinsed thoroughly with sterile water. The sterilized seeds were germinated in water at 28℃ in darkness and then grown in plastic boxes with water-soaked cotton in a growth chamber at 28℃ under a 16 h light/8 h dark regime. For suspension cultures, sterilized rice seeds were plated on NB medium supplemented with 2.0 mg/L 2, 4-D and were cultured in the dark at 28℃ for 4 weeks, as described by Kim et al. (2003). To establish embryogenic suspension-cultured cells, calli induced from embryos were transferred into 100 ml flasks containing liquid R2 medium supplemented with 2.0 mg/L 2, 4-D and 3% (w/v) sucrose (Ohira et al. 1973). Liquid cultures were maintained by weekly subculturing with shaking at 90-100 rpm. Log phase cells sampled 4 days after subculturing were used for RNA analysis.

Preparation of fungal elicitor

Mycelia of the rice blast fungus (*Magnaporthe grisea* KJ301), which is avirulent in the Milyang 117 variety of rice, were cultured at 25℃ for 5 days in a liquid medium (Potato Dextrose Broth). The mycelia were then minced and homogenized in 50 mM sodium acetate buffer, pH 4.5, sonicated, and centrifuged. The pellet was homogenized again in 0.1 M borate buffer, pH 8.8. The homogenate was then autoclaved at 121℃ for 10 min and centrifuged. The resulting supernatant was extensively dialyzed against distilled water at 4℃ and then freeze-dried (Doke and Furuchi, 1982). Reducing sugar content was determined by the dinitrosalicylic acid method (Dygert et al. 1965) using glucose as a standard.

Treatments with stress signals and chemicals

To examine the inducibility of the *Oshin1* gene in suspension-cultured rice cells, culture samples were treated with fungal elicitor (50 μg of glucose equivalents/ml), 2 mM hydrogen peroxide (H_2O_2), 1 mM salicylic acid (SA), 100 μM jasmonic acid (JA), 100 μM abscisic acid (ABA), calcium-ionophore (10 mM Ca2+/20 μM A23187), 100 μM BayK8644, 100 μM 2,5-di-tert-butylhydroquinone (BHQ), or 10 μM mastoparan. In some cultures, the following inhibitors were added to the cultures 1 h before fungal elicitor application: 2 mM BAPTA, 100 μM BAPTA/AM, 10 mg/ml cyclosporine A, and 200 μM W-7. Fungal elicitor and 5 mM ethephon were sprayed onto 3-week-old plants placed in plastic boxes, which were then sealed to prevent ethylene gas from escaping. After the treatments and incubations, samples were collected at the indicated time points for total RNA extraction. The chemicals were prepared in dimethylsulfoxide (DMSO) or water. For controls, total RNA was isolated from water or DMSO-treated samples.

RNA isolation and Northern blot analysis

Total RNA was isolated by phenol/chloroform extraction, followed by lithium chloride precipitation as described by Lagrimini et al. (1987). Total RNA (20 μg) was denatured and separated by electrophoresis on a 1.5% (w/v) agarose-formaldehyde gel, and transferred onto a nylon membrane (GeneScreen Plus, NEN, Boston). The membrane was prehybridized for 30 min and incubated with the ^{32}P-labeled full length *Oshin1* cDNA for 12-16 h at 60-65℃ in Church's hybridization solution (0.5 M Na_2HPO_4, pH 7.2, 1 mM EDTA, 1% BSA, 7% SDS) according to the method of Church and Gilbert (1984). The membranes were washed three times for 15 min each in 2 × SSC with 0.1% SDS at 60-65℃ and three times in 0.2 x SSC with 0.1% SDS at 60-65℃ for 15 min each. The blots were exposed to Kodak XAR-5 X-ray film at -80℃. Equal sample loading was confirmed by ethidium bromide staining of the samples in the gel and checked by hybridization of the blot with a probe recognizing the rice 18S ribosomal RNA (rDNA).

Southern blot analysis

Rice genomic DNA was isolated from fresh leaf tissues as described by Dellaporta et al. (1983). A 10 μg aliquot of genomic

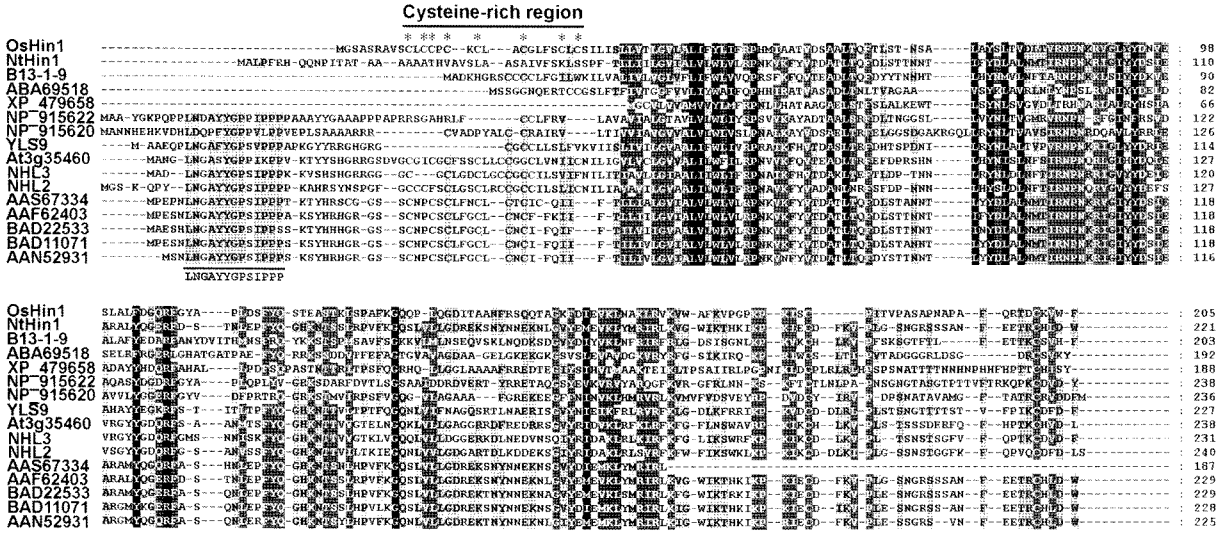

Figure 1. Comparison of the deduced amino acid sequence of *Oshin1* with other plant *hin1* homologues. Plant *hin1* homologues were obtained from a GenBank database search. The predicted *Oshin1* sequence is aligned with *hin1* homologues from tobacco (NtHin1, CAA 68848; BAD22533, AAF62403), potato (AAN52931, AAS67334), pepper (BAD11071), soybean (B13-1-9, BAB86894), Arabidopsis (NHL2, At3g11650; NHL3, At5g06320; YLS9, At2g35980; At2g35460), and rice (NP_915620, NP_915622, XP_479658, ABA96518). The Genedoc version 2.6 software (www.psu.edu/biomed/ genedoc) was used for alignment of amino acid sequences. The conserved amino acid residues with 100, 80, and 60 % conservation are shaded in black, dark gray, and light gray, respectively. Dashes indicate gaps introduced to maximize homology. A cysteine-rich domain in the N-terminal region in *Oshin1* is indicated by the asterisks. A large number of leucine (24 residues), serine (18 residues), and alanine (23 residues) residues are found in *OsHin1*

DNA was digested with restriction endonucleases (*Hind* III, *Eco*RI and *Xba* I), fractionated on a 0.8% (w/v) agarose gel, and transferred onto a nylon membrane. The membrane was incubated with the ^{32}P-labeled full length *Oshin1* cDNA. Hybridization and washing were performed using the same conditions described for the Northern blot analysis.

Sequence analysis

Searches for sequence similarities were carried out using BLAST (Basic Local Alignment Search Tool) (Altschul et al. 1990) on the National Center for Biotechnology Information (NLM, NIH, Bethesda, MD) server. The data analyses were performed using the Blastx or Blastn algorithms.

Results

Isolation of the *Oshin1* cDNA

To identify blast fungal elicitor-responsive genes in rice, we used an mRNA differential display technique and isolated a large number of fungal elicitor-responsive genes by screening a λZAP cDNA library, which was constructed from suspension-cultured rice cells treated with fungal elicitor (Kim et al. 2000). Suspension-cultured cells were treated with fungal elicitor from *M. grisea* for 30 min or 60 min. Total RNA was extracted from treated and untreated cell cultures and used for mRNA differential display experiments. From cDNA library screening we initially isolated 70 fungal elicitor-induced cDNAs and further characterized one clone, F20209. The F20209 clone is 1,067 bp long and shares significant sequence similarity with the tobacco *hin1* gene, which is rapidly activated by both harpins and avrPto-mediated signals (Gopalan et al. 1996). Therefore, we conclude that F20209 represented a putative *Oshin1* cDNA clone. The complete DNA sequence was submitted to the Genbank database (Accession Number AF039532). The putative *Oshin1* cDNA has an open reading frame encoding 205 amino acid residues with a basic isoelectric point of 8.9. The deduced protein sequence has a cysteine-rich region (ClCCpCkClaCglfsClC) at the N-terminus and is rich in leucine, serine, and alanine residues. The deduced amino acid sequence of *Oshin1* was compared to protein sequences deposited in the public databases using the NCBI Blastp program (Figure 1). *Oshin1* showed a significant sequence identity to other plant hin1-like proteins as shown in Figures 1 and 2. The *Oshin1* protein sequence lacks the N-terminal conserved region (-LNGAYYGPSIPPP-) found in most plant *hin1* homologues. Through sequence analysis, a

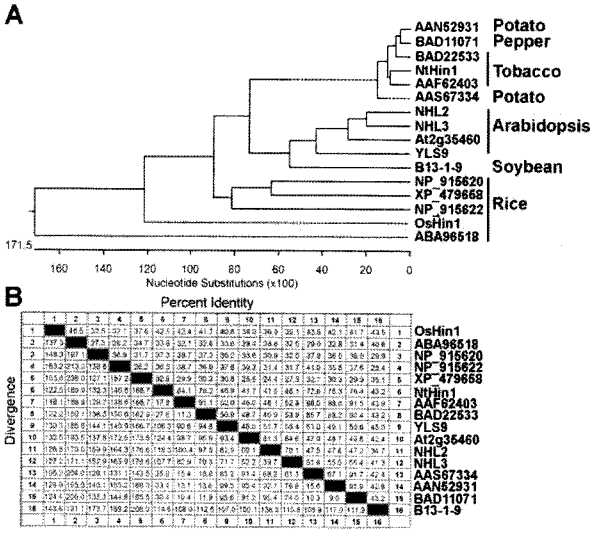

Figure 2. Phylogenetic analysis and sequence identity of plant *hin1* homologues. Plant *hin1* homologues were obtained from a GenBank database search. (A) The sequences were aligned and compared to generate a phylogenetic tree. (B) Sequence identities were determined by the ClustalW method using the MegAlign 5.0 program (DNASTAR Inc.)

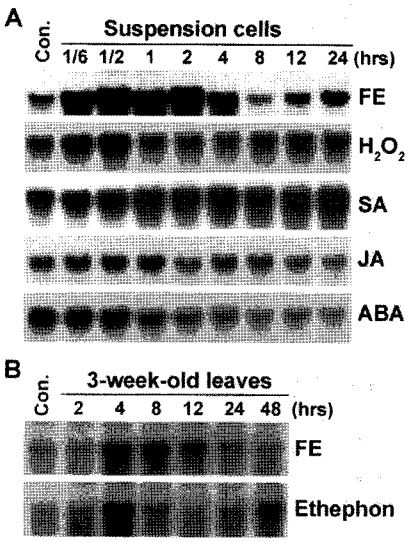

Figure 3. Expression patterns of the *Oshin1* transcript in response to various stimuli. Northern blot analysis of *Oshin1* expression induced by external stimuli in suspension-cultured rice cells (A) and 3-week-old leaves (B). The cells or 3-week-old plants were treated with fungal elicitor (FE, 50 μg ml-1 glucose equivalents), H2O2 (2 mM), SA (1 mM), JA (100 μM), ABA (100 μM), or ethephon (5 mM) and samples were harvested at the indicated time points. Total RNA (20 μg) was used for Northern blot analysis. The 32P-labeled full length *Oshin1* cDNA was used as a probe. The control is total RNA from water-treated samples

phylogenetic tree was generated by Clustal W using the MegAlign 5.0 program (DNASTAR Inc.). The phylogenetic tree shows that *Oshin1* is divergent from other plant *hin1* family genes, but showed a slightly higher similarity to other Hin1-like proteins from rice (Figure 2A). Sequence identity at the amino acid level (32.1 to 46.5%) is shown in Figure 2B. The NCBI database search revealed that the *Oshin1* gene is located on chromosome #4 of the rice genome (japonica and indica cultivars). Comparison of the *Oshin1* cDNA sequence with the genomic sequence showed that there are no introns in the *Oshin1* gene.

Analysis of *Oshin1* gene expression in response to various stress treatments

In our previous report, we demonstrated that *Oshin1* transcript levels are specifically elevated upon infection with an avirulent rice blast fungal pathogen (Kim et al. 2000). In order to further study the expression patterns of the *Oshin1* transcript under environmental stress conditions such as fungal elicitor, H_2O_2, SA, JA, and ABA, the level and time course of *Oshin1* expressions was examined by Northern blot analysis using total RNA isolated from both rice cell suspension cultures and 3-week-old leaf tissues (Figure 3). The expression level of the *Oshin1* transcript increased as early as 10 min and reached a maximum level at 2 h after treatment with fungal pathogen elicitor. The *Oshin1* transcript was also rapidly and highly induced within 30 min in response to H_2O_2, and decreased after 1 h of treatment. SA treatment caused a steady state upregulation of *Oshin1* expression for up to 24 h. However, the plant hormones JA and ABA induced a decrease in *Oshin1* expression from 1 to 2 h after such treatments. In addition, the *Oshin1* transcript was induced by fungal pathogen elicitor in 3-week old rice leaf tissues. Ethephon, a precursor of the gaseous plant hormone ethylene, induced an effective elevation in the *Oshin1* transcript 4 to 8 h after treatment in 3-week old leaves (Figure 3B). These results suggest that *Oshin1* expression is induced by multiple defense/stress response pathways.

Induction of the *Oshin1* transcript by calcium signals

Since cytosolic free calcium is elevated in plant cells in response to a variety of external stimuli, we examined whether *Oshin1* expression is induced by calcium signals. Treatment of suspension-cultured cells with calcium-ionophore (Ca^{2+}/A23187) in the absence of fungal elicitor increased the expression of *OsHin1*. As shown in Figure 4A, the *Oshin1* transcript was induced to a high level within 10 min by treatment with Ca^{2+}/A23187 and decreased after 4 h. Since the

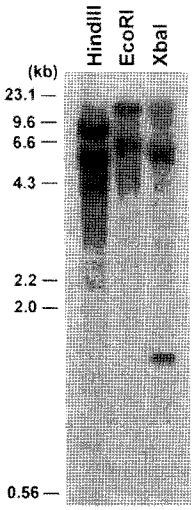

Figure 5. Genomic Southern blot analysis of the *Oshin1* gene in the rice genome. A 10 μg aliquot of genomic DNA was digested with EcoRI, XbaI, and HindIII restriction enzymes, separated on a 0.8% (w/v) agarose gel and transferred onto a nylon membrane. The membrane was hybridized with a full-length 32P-labeled *Oshin1* cDNA probe. The membrane was washed three times for 10 min in 2 x SSC with 0.1% SDS and three times in 0.2 x SSC with 0.1% SDS at the 60-65 ‡C. The DNA size markers are shown in kb on the left

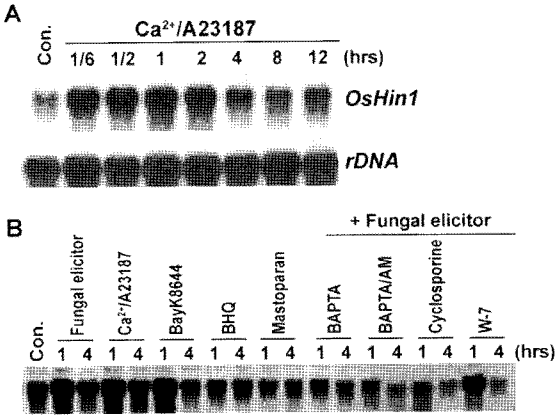

Figure 4. Induction of *Oshin1* transcript by calcium signals. (A) Northern blot analysis of *Oshin1* expression following treatment with Ca^{2+}-ionophore (10 mM CaCl2 and 20 μM A23187) in the cell cultures. (B) Effects of various inhibitors on the induction of *Oshin1* transcript in response to the fungal elicitor. Inhibitors were added 1 h prior to treatment of rice suspension cells with fungal elicitor and samples were taken at 1 and 4 h after treatment with fungal elicitor. The inhibitor treatments were10 mM Ca2+/20 μM A23187, 100 μM BayK8644 (calcium channel activator), 100 μM BHQ (Ca2+-ATPase inhibitor), 10 μM mastoparan (increases intracellular Ca2+ levels), 2 mM BAPTA (extracellular Ca2+ chelator), 100 μM BAPTA/AM (intracellular Ca2+ chelator), 10 mg/mL cyclosporine A (calcineurin inhibitor), or 200 μM W-7 (calmodulin antagonist) dissolved in DMSO. The control is the total RNA from DMSO-treated samples

expression of *Oshin1* was induced by Ca^{2+}/A23187, the effect of chemicals mobilizing the intracellular Ca^{2+} level on the expression of *Oshin1* was further examined in suspension-cultured cells. To gain further insight into the potential molecular mechanism mediating *Oshin1* induction in response to calcium ions and fungal elicitor, pharmacological studies were performed with various inhibitors and analyzed by Northern blot (Figure 4B). Application of the calcium channel activator BayK8644, which stimulates an elevation in cytosolic Ca^{2-}, induced the expression of *OsHin1*, similar to the response measured with Ca2+/A23187 treatment. An inhibitor of the Ca^{2+}-ATPase, 2,5-di-tert-butylhydroquinone (BHQ), and mastoparan, a compound which elevates intracellular Ca2+, did not affect the induction of *OsHin1*. Interestingly, inhibition of an external Ca^{2+} influx by the application of extracellular and intracellular Ca^{2+} chelators, BAPTA and BAPTA/AM, greatly reduced the expression of *Oshin1* induced by fungal elicitor. Furthermore, cyclosporine A, a protein phosphatase 2B inhibitor, blocked the expression of *Oshin1* by fungal elicitor, and W-7, a calmodulin antagonist, slightly diminished its expression. These results suggest that an influx of Ca^{2+} ions plays a crucial role in the elicitor-mediated expression of *Oshin1* and that dephosphorylation by protein phosphatase 2B participates in this process.

Genomic Structure of *Oshin1*

In order to determine the copy number of the *Oshin1* gene in the rice genome, genomic Southern blot analysis was carried out. Rice genomic DNA was digested with three different restriction endonucleases, *Hind* III, *EcoR* I, and *Xba* I. These restriction enzymes were not present in the *Oshin1* cDNA sequence. The digested DNA was hybridized with a ^{32}P-labeled full-length cDNA probe of *OsHin1*. As shown in Figure 5, the *Oshin1* probe hybridized with at least two restriction digested DNA fragments, indicating the presence of closely related genes in the rice genome. Therefore, the *Oshin1* gene is likely to be a member of the rice *hin1* gene family. In deed, a database analysis uncovered several *hin1* homologues in rice.

Discussion

We previously isolated a number of fungal elicitor-responsive genes in rice using an mRNA differential display technique. Some elicitor-responsive genes, including *OsHin1*, were induced specifically in response to an avirulent infection of rice cells with the rice blast fungus (Kim et al. 2000). We observed that these elicitor-responsive

genes were inducible by various other environmental stimuli as well (Kim et al. 1999; Kim et al. 2003; Lee et al. 2003). These stimuli are therefore useful as potential molecular probes to study elicitor-mediated signal transduction pathways. We show here that *Oshin1* is involved in both biotic and abiotic stress responses since its expression is activated by a variety of environmental stresses, such as the blast fungal elicitor, H2O2, SA, ethephon, and Ca^{2+}/A23187 (Figure 3). These are potential signaling molecules involved in the regulation of a number of defense genes (Raymond and Farmer 1998).

Previously, we observed that the expression of the *Oshin1* transcript is specifically elevated upon infection of suspension-cultured rice cells with an avirulent pathogen (Kim et al. 2000). Through a database analysis, we found that there are several *hin1* gene homologues in various plants. The tobacco *hin1* gene is a valuable molecular marker in plant HR defense responses (Gopalan and He 1996; Pontier et al. 1999; Varet et al. 2002). In addition, tobacco *hin1* is induced both during HR and during leaf senescence (Pontier et al. 1999). These data suggested that cross-talk between those two different cell death signaling pathways might exist. It will be interesting to examine whether *Oshin1* can be induced during leaf senescence. The expression of the *Oshin1* transcript was elevated by ethylene treatment, as shown in Figure 3, and this result raises the possibility that expression of *Oshin1* is enhanced in response to leaf senescence since ethylene is a key hormone in regulating the onset of leaf senescence (Zacarias and Reid 1990; Grbi and Bleecker 1995). Furthermore, the Arabidopsis *hin1* homologue YLS9 is most abundant in senescent leaves (Yoshida et al., 2001). Ethylene and ABA induced an increase in YLS9 expression. Varet et al. (2002) reported that the Arabidopsis genome contains 28 genes with sequence homology to the Arabidopsis *NDR1* and the tobacco *hin1* genes, and named them *NDR1/HIN1*-like genes. Among these genes, *NHL25* and *NHL3* were induced specifically during infection with an avirulent pathogen. SA treatment also induced expression of *NHL25* and *NHL3*. Theses results suggest that *hin1* homologues including *Oshin1* are involved in plant defense responses against various environmental stresses.

Rakwal et al. (2004) reported the transcriptional profiling of *Oshin1* in 2-week-old seedlings (cv. Nipponbare). Their report indicated that *Oshin1* was not expressed in leaf segments treated with the fungal elicitor chitosan. Pathogen infection with rice blast fungus also did not induce *Oshin1* expression. In addition, JA, SA, H_2O_2, ABA, ethylene, and a heavy metal treatment ($CuSO_4$) failed to induce *Oshin1* expression. However, wounding (by cutting) strongly up-regulated *Oshin1* expression in leaf segments, which was further enhanced by application of protein phosphatase inhibitors. This study also suggested that *Oshin1* expression follows a circadian rhythm with highest expression during the dark period. Further study, however, is required to determine whether or not *Oshin1* is a circadian gene. In contrast, our present findings on *Oshin1* expression in suspension cultured cells showed distinct upregulation of *Oshin1* in response to fungal elicitor, H_2O_2, and SA. Additionally, we found that *Oshin1* is upregulated in response to the application of ethephon and fungal elicitor in 3-week-old leaf tissues. These differences may be due to differences in penetration efficiency of the inducer molecules, cell type dependence, or tissue specificity of *Oshin1* expression between plant leaves and suspension cultured cells. In deed, we noted previously that the rice chitinase gene *Rcht2* responded differently to fungal elicitor in suspension cultured cells versus leaf tissues (Kim et al., 1998). The *Rcht2* transcript level in suspension cultured cells was strongly increased by treatment with fungal elicitor, whereas *Rcht2* expression was not induced in rice leaves treated with fungal elicitor. Since there are technical difficulties in the uniform treatment of whole plants with stressing reagents, we used a rice cell suspension culture to study the induction of *OsHin1*. Model systems employing suspension-cultured plant cells and elicitor preparations isolated from pathogens have proved extremely useful in identifying the biochemical and physiological aspects of the defense response and in isolating putative plant defense-related genes. Treatment of suspension-cultured cells from parsley, soybean, and tobacco with a fungal elicitor from *Phytophthora sojae* caused ion fluxes, an oxidative burst, phytoalexin production, and activation of defense-related genes (Ebel et al. 1994; Nürnberger et al. 1994; Rusterucci et al. 1996; Yu 1995). Similar responses have also been described for suspension-cultured tomato cells treated with elicitor preparations derived from different microorganisms such as yeast, *Pseudomonas syringae*, and *Cladosporium fulvum*.

In our suspension cultured rice cell system, we found that the expression of *Oshin1* in response to fungal elicitor is blocked by pretreatment of the cells with a protein phosphatase 2B (calcineurin) inhibitor, suggesting that protein phosphatase 2B dependent dephosphorylation events are involved in the elicitor-mediated induction of *Oshin1* and might be required for the induction of *Oshin1* expression in plant defense responses. Thus, it will be interesting to identify signaling molecules mediating phosphorylation/dephosphorylation

events in modulating *Oshin1* expression in the elicitor-mediated signal transduction pathways.

On the basis of our results for the *Oshin1* gene and studies of other *hin1* family genes, we suggest that *Oshin1* is involved in defense/stress responses against multiple external stimuli, although further studies will be needed to clarify its role. Defining the exact role of *Oshin1* during defense responses to environmental stimuli in rice will provide valuable insight into the elicitor-signal transduction pathways implicated in disease resistance.

Acknowledgements

This work was supported by a grant from KOSEF to the Environmental Biotechnology National Core Research Center (grant #: R15-2003-012-02003-0), by a grant from 'BK21' Program from the Ministry of Education and Human Resources Development, and by a grant from the KRIBB Research Initiative Program.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403-410

Church GM, Gilbert W (1984) Genomic sequencing. Proc Natl Acad Sci USA 81:1991-1995

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19-21

Dixon RA, Lamb CJ (1990) Molecular communication in interactions between plant and microbial pathogens. Annual Rev Pl Physiol Pl Molec Biol 41:339-367

Doke N, Furuichi N (1982) Response of protoplasts to hyphal wall components in relationship to resistance of potato to *Phytophthora infestans*. Plant Pathol 2:23-30

Dygert SL, Li LH, Florida D, Thoma J (1965) Determination of reducing sugar with improved precision. Anal Biochem 13: 367-374

Ebel J, Bhagwat A, Cosio EG, Feger M, Kissel U, Mithöfer A, Waldmuller T (1994) Elicitor-binding proteins and signal transduction in the activation of a phytoalexin defense response. Can J Bot 37:S506-S510

Gopalan S, Wei W, He SY (1996) *hrp* gene-dependent induction of *hin1*: a plant gene activated rapidly by both harpins and *avrPto* gene-mediated signal. Plant J 10:591-600

Grbi V, Bleecker AB (1995) Ethylene regulates the timing of leaf senescence in *Arabidopsis*. Plant J 8:595-602

Kim CY, Gal SW, Choe MS, Jeong SY, Lee SI, Cheong YH, Lee SH, Choi YJ, Han C-d, Kang KY, Cho MJ (1998) A new class II rice chitinase, *Rcht2*, whose induction by fungal elicitor is abolished by protein phosphatase 1 and 2A inhibitor. Plant Mol Biol 37:523-534

Kim WY, Kim CY, Cheong NE, Choi YO, Lee KO, Lee S-H, Park JB, Nakano A, Bahk JD, Cho MJ, Lee SY (1999) Characterization of two fungal-elicitor-induced rice cDNAs encoding functional homologues of the rab-specific GDP-dissociation inhibitor. Planta 210:143-149

Kim CY, Koo YD, Jin JB, Moon BC, Kang CH, Kim ST, Park BO, Lee SY, Kim ML, Hwang I, Kang KY, Bahk JD, Lee SY, Cho MJ (2003) Rice C2-domain proteins are induced and translocated to the plasma membrane in response to a fungal elicitor. Biochemistry 42:11625-11633

Kim CY, Lee S-H, Park HC, Bae CG, Cheong YH, Choi YJ, Han CD, Lee SA, Lim CO, Cho MJ (2000) Identification of rice blast fungal elicitor-responsive genes by differential display analysis. Mol Plant-Microbe Interact 13:470-474

Lagrimini LM, Burkhart W, Moyer M, Rothstein S (1987) Molecular cloning of complementary DNA encoding the lignin-forming peroxidase from tobacco: Molecular analysis and tissue-specific expression. Proc Natl Acad Sci USA 84:7542-7546

Lee WY, Hong JK, Kim CY, Chun HJ, Park HC, Kim JC, Yun DJ, Chung WS, Lee S-H, Lee SY, Cho MJ, Lim CO (2003) Over-expressed rice ADP-ribosylation factor 1 (RARF1) induces pathogenesis-related genes and pathogen resistance in tobacco plants. Physiologia Plantarum 119:573-581

Nürnberger T, Nennstiel D, Jabs T, Sacks WR, Hahlbrock K, Scheel D (1994) High affinity binding of a fungal oligopeptide elicitor to parsley plasma membranes triggers multiple defense responses. Cell 78:449-460

Ohira K, Ojima K, Fujiwara A (1973) Studies on the nutrition of rice cell culture. I. A simple, defined medium for rapid growth in suspension culture. Plant Cell Physiol 14:1113-1121

Pontier D, Gan S, Amasino RM, Roby D, Lam E (1999) Markers for hypersensitive response and senescence show distinct patterns of expression. Plant Mol Biol 39:1243-1255

Rakwal R, Agrawal GK, Tamogami S, Iwahashi H (2004) Transcriptional profiling of *Oshin1* in rice plants: a potential role in defense/stress and development. Plant Sci 166:997-1005

Raymond P, Farmer EE (1998) Jasmonate and salicylate as global signals for defense gene expression. Curr Opin Plant Biol 1:404-411

Rustérucci C, Stallaert V, Milat ML, Pugin A, Ricci P, Blein JP (1996) Relationship between active oxygen species, lipid peroxidation, necrosis, and phytoalexin production induced by elicitins in *Nicotiana*. Plant Physiol 111:885-891

Ryan CA, Farmer EE (1991) Oligosaccharide signals in plants: a current assessment. Annu Rev Plant Physiol Plant Mol Biol 42:651-674

Varet A, Parker JE, Tornero P, Nass N, Nürnberger T, Dangl JL, Scheel D, Lee J (2002) NHL25 and NHL3, two NDR1/HIN1-like genes in *Arabidopsis thaliana* with potential role(s) in plant defense. Mol Plant-Microbe Interact 15:608-616

Wei ZM, Laby RJ, Zumoff CH, Bauer DW, He SY, Collmer A,

Beer SV (1992) Harpin, elicitor of the hypersensitive response produced by the plant pathogen *Erwinia amylovora*. Science 257:85-88

Willis DK, Rich JJ, Hrabak EM (1991) *hrp* genes of phytopathogenic bacteria. Mol Plant-Microbe Interact 4:132-138

Yoshida S, Ito M, Nishida I, Watanabe A (2001) Isolation and RNA gel blot analysis of genes that could serve as potential molecular markers for leaf senescence in *Arabidopsis thaliana*. Plant Cell Physiol 42:170-178

Yu LM (1995) Elicitins from *Phytophthora* and basic resistance in tobacco. Proc Natl Acad Sci USA 92:4088-4094

Zacarias L, Reid MS (1990) Role of growth regulators in the senescence of *Arabidopsis thaliana* leaves. Physiologia Plantarum 80:549-554

(Received March 10, 2009; Accepted March 17, 2009)