Plant Cell Rep (2012) 31:1845–1850
DOI 10.1007/s00299-012-1298-9

ORIGINAL PAPER

Over-expression of rice leucine-rich repeat protein results in activation of defense response, thereby enhancing resistance to bacterial soft rot in Chinese cabbage

Young Ho Park · Changhyun Choi · Eun Mi Park · Hyo Sun Kim · Hong Jae Park · Shin Cheol Bae · Ilpyung Ahn · Min Gab Kim · Sang Ryeol Park · Duk-Ju Hwang

Received: 17 April 2012 / Revised: 4 June 2012 / Accepted: 4 June 2012 / Published online: 21 June 2012
© Springer-Verlag 2012

Abstract *Pectobacterium carotovorum* subsp. *carotovorum* causes soft rot disease in various plants, including Chinese cabbage. The simple extracellular leucine-rich repeat (eLRR) domain proteins have been implicated in disease resistance. Rice leucine-rich repeat protein (OsLRP), a rice simple eLRR domain protein, is induced by pathogens, phytohormones, and salt. To see whether OsLRP enhances disease resistance to bacterial soft rot, OsLRP was introduced into Chinese cabbage by *Agrobacterium*-mediated transformation. Two independent transgenic lines over-expressing OsLRP were generated and further analyzed. Transgenic lines over-expressing OsLRP showed enhanced disease resistance to bacterial soft rot compared to non-transgenic control. Bacterial growth was retarded in transgenic lines over-expressing OsLRP compared to non-transgenic controls. We propose that OsLRP confers enhanced resistance to bacterial soft rot. Monitoring expression of defense-associated genes in transgenic lines over-expressing OsLRP, two different glucanases and *Brassica rapa* polygalacturonase inhibiting protein 2, PDF1 were constitutively activated in transgenic lines compared to non-transgenic control. Taken together, heterologous expression of OsLRP results in the activation of defense response and enhanced resistance to bacterial soft rot.

Communicated by J. S. Shin.

Y.H. Park and C. Choi contributed equally to this work.

Y. H. Park · C. Choi · E. M. Park · H. S. Kim · H. J. Park · S. C. Bae · I. Ahn · M. G. Kim · S. R. Park · D.-J. Hwang (✉)
National Academy of Agricultural Science, RDA, Suwon 441-707, Korea
e-mail: djhwang@rda.go.kr

Keywords Rice leucine-rich repeat protein · Disease resistance · Bacterial soft rot · Chinese cabbage

Abbreviations

OsLRP	*Oryza sativa* leucine-rich repeat protein
Pcc	*Pectobacterium carotovorum* subsp. *carotovorum*
HR	Hypersensitive response
HIR	Hypersensitive-induced reaction
Xoo	*Xanthomonas oryzae* pv. *oryzae*

Introduction

Brassica rapa L. subsp. *pekinensis* (Chinese cabbage) is one of the most consumed and important vegetables cultivated across 37,200 ha in 2009 in Korea. Bacterial soft rot is the most severe and destructive disease in most vegetables, including Chinese cabbage. Chinese cabbage is highly susceptible to soft rot disease caused by Gram-negative bacterium, *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*) (Ren et al. 2001). In addition, control of soft rot disease is difficult due to a wide range of hosts, the ability of the bacteria to survive in plant debris in the soil, and host susceptibility (Vanjildorj et al. 2009). Chemical controls are not currently available for bacterial soft rot.

Pcc causes maceration of parenchymal tissue by increasing the levels of multiple exoenzymes including pectinases, cellulases, and proteases in all organs, ultimately resulting in plant cell death. These cell wall degrading enzymes break pectin down into unsaturated oligogalacturonates and trigger various plant defense responses, including phytoalexin synthesis, proteinase inhibitor synthesis, membrane protein phosphorylation, and

a response similar to a hypersensitive response (HR) (Lam et al. 2001; Vlot et al. 2008; Ban et al. 2009).

Plant proteins with extracellular leucine-rich repeat (eLRR) domains are classified into five classes based on their domain organization: polygalacturonase inhibitor protein (PGIP)-like proteins, leucine-rich repeat-extensin-like proteins, receptor-like proteins, receptor-like kinases, and simple eLRR domain proteins (van der Hoorn et al. 2005; Zhou et al. 2009). Simple eLRR domain proteins have been reported in several plant species, such as sorghum, tomato, tobacco, *Arabidopsis*, and rice (Hipskind et al. 1996; Tornero et al. 1996; Jacques et al. 2006; Jung and Hwang 2007). It is proposed that the sorghum eLRR (SLRR) protein may be involved in protein–ligand binding (Hipskind et al. 1996). The tomato eLRR (LeLRP) protein is induced in diseased plants and is processed during pathogen infection (Tornero et al. 1996). The tobacco eLRR (NtLRP1) protein is localized in the endoplasmic reticulum and proposed to be a negative regulator of the elicitin-mediated HR (Jacques et al. 2006). The pepper eLRR (CaLRR1) protein is induced by pathogens and expressed in the phloem of *Capsicum annuum* cells (Jung et al. 2004). The CaLRR1 protein interacts with hypersensitive-induced reaction (HIR) protein, CaHIR1, and suppresses cell death induced by CaHIR1 (Jung and Hwang 2007). Recently, it was demonstrated that the rice eLRR protein (OsLRR1) enters the endosomal pathway and interacts with OsHIR1. Over-expression of OsLRR1 in *Arabidopsis* confers enhanced resistance to the biotrophic pathogen, *Pseudomonas syringae*, and causes the constitutive expression of defense-related genes, such as PR1, PR2, and PDF1 (Zhou et al. 2009). More recently, OsLRR1 was found to enhance the plasma membrane localization of OsHIR1 (Zhou et al. 2010).

We have been interested in rice-*Xanthomonas oryzae* pv. *oryzae* (*Xoo*) interaction. We isolated the *Oryza sativa* LRR protein (OsLRP) (GenBank accession number AF364178) from expressed sequence tag (EST) analysis of a cDNA library made with mRNAs from *Xoo*-infected leaves. While we were doing research on OsLRP in rice, a paper on OsLRR1, the same gene as OsLRP, was published as mentioned above. Therefore, we introduced OsLRP into Chinese cabbage to develop a crop resistant to bacterial soft rot in this study. Transgenic plants were analyzed and challenged against the bacterial soft-rot pathogen, *Pcc*.

Materials and methods

Plant treatments

Rice seedlings (*Oryza sativa* cv. Hwachung) were grown in a greenhouse for 3 weeks. Three-week-old rice seedlings were washed and incubated in tap water for 2 days and then treated with 1 mM benzothiadiazole (BTH), 1 mM salicylic acid (SA), 100 μM jasmonic acid (JA), 200 mM NaCl, 100 μM abscisic acid (ABA), respectively. Samples were taken at indicated times in Fig. 1. A strain of *Xoo* KXO98 incompatible to *O. sativa* cv. Hwachung was grown in PSA medium (10 g peptone, 10 g sucrose, 1 g sodium glutamate, and 15 g agar per liter) for 2 days and then resuspended in 10 mM $MgCl_2$ to a final OD_{600} of 0.5. For bacterial inoculations, 21-day-old rice seedlings were challenged by spraying bacterial suspension with atomizer (Ji et al. 2008). Samples were taken at 12, 24, 48 h post-inoculation (hpi) and then were immediately frozen in liquid nitrogen and stored at −80 °C until further analysis.

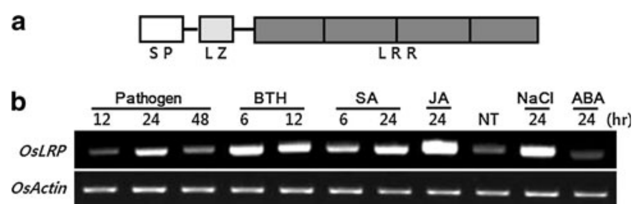

Fig. 1 Schematic representation of *Os*LRP domains and expression analysis of *OsLRP1* in response to various stimuli. **a** *Os*LRP contains a signal peptide (SP), a leucine zipper (LZ), and a LRR domain with four leucine-rich repeats. **b** RT-PCR using rice leaves treated with pathogen (*Xoo*), phytohormones (SA, JA, and ABA), BTH, NaCl, and non-treated control (NT) was performed. *Os*Actin was used for normalization of each sample. Each treatment is described in "Materials and methods"

Reverse transcription-polymerase chain reaction (RT-PCR)

Leaf samples were ground to powder in liquid nitrogen and total RNAs were prepared for RT-PCR as previously described (Hwang et al. 2008). Total RNA (1 μg) was used for reverse transcription with M-MLV RTase (Promega, Madison, WI, USA). Two microliters of each reverse transcriptase reaction was used for subsequent PCR. The PCR reaction was performed with 28 cycles (95 °C for 30 s, 55–60 °C for 30 s and 72 °C for 30 s) and a final extension for 5 min at 72 °C. The actin gene primer pair (BrActin or OsActin) was used as a control for the same amount of transcripts from all samples in this experiment. The primers used in this study are listed in Table 1.

Isolation of *OsLRP* and plant transformation vector construction

Full-length cDNA corresponding to *OsLRP* gene was obtained by EST analysis of a cDNA library made with mRNAs from *Xoo*-infected leaves. *OsLRP* gene-specific primers were designed from the sequence of GenBank

Table 1 Primers used in this study

Gene	Accession no.	Sequence (5′–3′)	Experiment
OsLRP	AF364178.1	F: AAAAAGCAGGCTTCATGGGGGCGGGGGCG	Vector construction
		R: AGAAAGCTGGGTACTAGCAGTTGGTGTC	
		F: TGGGAAATTAACGTCCCTTG	RT-PCR
		R: CCAGGCCTTGTAGTTCTGGA	
OsActin	XM469569	F: TCCATCTTGGCATCTCTCAG	RT-PCR
		R: GTACCCGCATCAGGCATCTG	
MERI5B	EX109664.1	F: TCTGGAACCCTCAAGAATCATATG	RT-PCR
		R: CATAGGATAGAAAGAAAGCAGAGG	
Meri-5	EX114801.1	F: TCTCGGGGTTTTGTTTCCAAAGAG	RT-PCR
		R: ACACCCTTTGCTTTATTGGACAAC	
BrActin	Lee (2005)[a]	F: CGGTCCAGCTTCGTCATACTCAGCC	RT-PCR
		R: AAATGTGATGTGGATATCAGGAAGG	
BrPGIP2	FJ771027	F: GCCAAGCTCAAGTATCTCCGGAGC	RT-PCR
		R: ACACTTGTTGTGAAAATAGGAATAAGTATCGAATTC	
PDF1.2	AT5G44420	F:AGAAATATGCATGTCATAAAGTTACTCAT	RT-PCR
		R:CAATGGTGGAAGCACAGAAG	
hsr3	AJ131392	F: GATCACTGTGCAAGGAGTCTTCTTC	RT-PCR
		R: TAATCGAATCAATGATACCCAATTGGAGC	

The underlined sequences indicate the attB1 and attB2 sites for gateway vector construction

[a] Reference introducing the primer sequence

accession No. AF364178 (Table 1) for gateway cloning (Invitrogen, Carlsbad, CA, USA). PCR was performed with 30 cycles (94 °C for 30 s, 53 °C for 1 min, and 72 °C for 1 min) and a final extension at 72 °C for 7 min. The 922-bp product was cloned into pDONR221 to make an entry clone through the use of BP clonase (Invitrogen, Carlsbad, CA, USA). *35S:OsLRP* was made via LR clonase reaction between the *OsLRP* entry clone and pB2GW7 (Gateway™, Belgium). The map of resulting vector (*35S:OsLRP*) is shown in Fig. 2.

Transformation of Chinese cabbage

Seeds of Chinese cabbage (*Brassica rapa* subsp. *pekinensis* cv. Seoul) were sterilized with 70 % ethanol for 1 min, followed by treatment with 2 % sodium hypochlorite for 20 min. Seeds were placed on Murashige and Skoog (MS) medium containing 30 g/l sucrose and 6 g/l phytagel after washing with sterile distilled water. The plates were maintained at 25 °C under dark conditions for 6 days and transferred into light conditions for 1 day. The cotyledons were excised (1 cm) and placed on CO medium (MS medium containing sucrose 30 g/l, 1 mg/l 1-naphthalene-acetic acid, 5 mg/l 6-benzylaminopurine, 8 mg/l $AgNO_3$, and 8 g/l phytagel) for 3 days under light conditions. *Agrobacterium* (GV3101) carrying *35S-OsLRP*were grown overnight in 25 ml YEP medium containing 5 µg/ml

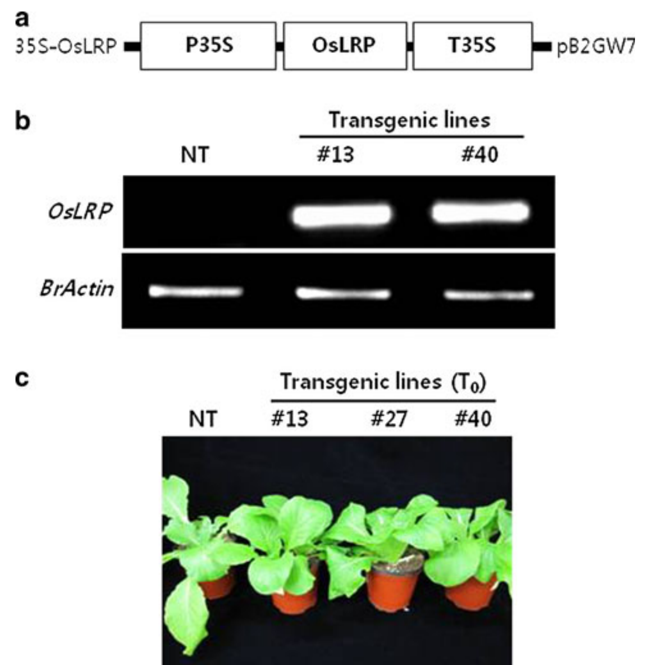

Fig. 2 Schematic representation of the *35S-OsLRP* construct for transformation into Chinese cabbage and the transcript level of *OsLRP* transgene in Chinese cabbage. **a** Linear map of the 35S-*OsLRP* construct for plant transformation. P35S: cauliflower mosaic virus 35S promoter; T35S: cauliflower mosaic virus 35S terminator. **b** RT-PCR was performed to analyze the expression pattern of the transgene in transgenic lines (T_0). **c** The photo shows phenotypes of T_0 transgenic lines and non-transgenic control (NT)

spectinomycin and harvested by centrifugation. The pellet was resuspended in 30 ml CO liquid media containing 12 mg acetosyringone, and then the cotyledon explants were added to the medium for 15 min under dark conditions. The explants were blotted onto sterile 3 M paper and then placed on CO medium for co-cultivation. After 3 days, the explants were rinsed three times in CO liquid medium containing 250 mg/l cefotaxime and then surface-dried on sterilized filter paper. For selection, the explants were transferred into SM medium (CO medium containing 250 mg/l cefotaxime and 5 mg/l L-phosphinothricin) and a 16/8 h day/night photoperiod for 6 weeks. The calli with multiple shoots on the SM medium were transferred to rooting medium (MS medium containing 30 g/l sucrose, 8 g/l phytagel, 250 mg/l cefotaxime, and 5 mg/l L-phosphinothricin). After 3 weeks, the root-plants were transferred to soil for acclimation.

Disease assay of transgenic Chinese cabbage *Pcc* stock was inoculated in 5 ml Luria–Bertani (LB) broth and were grown overnight in a 30 °C shaking incubator. The next day, the overnight culture was re-inoculated into fresh 5 ml LB broth in 1/100 dilution and were grown for 6 h under the same condition. After centrifugation of culture, the pellet was re-suspended in 1 mM NaCl to obtain an OD_{600} value of 0.1. The three leaves from 2-month-old non-transgenic plants (T_0 lines) were excised, respectively and placed onto petri dishes. Sterile toothpicks were used to make wound on the midribs of the leaves and 5 μl of the bacterial cell suspension was dropped onto the wound sites. The leaves were then transferred to a plastic box at 25 °C.

Transgenic plants (T_1) were challenged with *Pcc* at 10^3 cells/ml by infiltration with needless syringe. Three leaf discs from five plants (T_1) were harvested from each infiltration area with a 0.8-cm cork borer at 0 and 3 days post-inoculation (dpi), respectively. The discs were ground with a sterile mortar and pestle in 0.3 ml of 10 mM $MgCl_2$ and then diluted and plated on LB media containing 5 mg/ml rifampicin to determine bacterial population density (CFU/cm^2). The colonies were counted after the plates were incubated at 28 °C for 3 days. An average of bacterial numbers was made from numbers of colonies from samples of ten different T_1 plants and each error bar indicates standard deviation in the graph.

Results and discussion

Expression analysis of *OsLRP* in response to various stimuli

Initially, we isolated *OsLRP* by EST analysis of a cDNA library prepared from mRNAs of leaves infected with *Xoo*. OsLRP is also known as OsLRR1 (Zhou et al. 2009). The eLRR domain proteins, such as SLRR, LeLRR, NtLRP, CaLRR, and OsLRR1, have been implicated in defense responses to biotic stresses in various plant species (Hipskind et al. 1996; Tornero et al. 1996; Jung et al. 2004; Jacques et al. 2006; Jung and Hwang 2007; Zhou et al. 2009, 2010). *OsLRP* was previously reported to be induced by *Xoo* and wounding. To analyze the expression patterns of *OsLRP* in response to various stimuli, including *Xoo* infection, we carried out RT-PCR analysis (Fig. 1). *OsLRP* was induced by *Xoo* as previously reported (Zhou et al. 2009). In addition, *OsLRP* was up-regulated by biotic stimuli such as BTH, SA, and JA. *OsLRP* was also up-regulated by abiotic stress, such as salt, but not by ABA, suggesting that *OsLRP* may be involved in biotic stresses as well as abiotic stresses (Fig. 1).

Generation of transgenic Chinese cabbage lines

The simple eLRR domain proteins have been implicated in disease resistance. It is previously reported that over-expression of *OsLRR1* confers enhanced resistance to pathogen in *Arabidopsis* (Zhou et al. 2009). To develop Chinese cabbage resistance to bacterial soft rot, *OsLRP* was introduced into Chinese cabbage. The plant expression vector (*35S:OsLRP*) for *OsLRP* was made using the pB2GW7vector (Fig. 2) (Hwang et al. 2011). *OsLRP* is located downstream of 35S promoter. The *35S-OsLRP* construct was introduced into Chinese cabbage by *Agrobacterium*-mediated transformation. We obtained more than 10 independent regenerated plants. Finally, we obtained four independent transgenic lines by genomic PCR using bar-specific primers (data not shown). Two independent lines were further analyzed. To determine whether *OsLRP* is over-expressed in Chinese cabbage, we carried out RT-PCR. As shown in Fig. 2a, *OsLRP* was over-expressed in transgenic lines #13 and #40. *OsLRP* was not detected in non-transgenic controls. Transgenic plants over-expressing *OsLRP* showed normal phenotypes compared to non-transgenic controls (Fig. 2c).

Over-expression of *OsLRP* results in enhanced resistance to bacterial soft rot

Since over-expression of *OsLRP* resulted in enhanced resistance to *Pseudomonas syringae* pv. *syringae* in *Arabidopsis*, we tried to challenge T_0 transgenic plants with the soft-rot pathogen, *Pcc*. We made wounds by toothpicks and then dropped 5 μl of a cell suspension (OD_{600} of 0.1). Photos were taken at 1 day post-inoculation (dpi) (Fig. 3a). Transgenic lines #27 and #40 did not show soft-watery symptoms around infection sites, whereas non-transgenic controls showed severe soft-rot symptoms around infection sites. Transgenic plants over-expressing *OsLRP* conferred

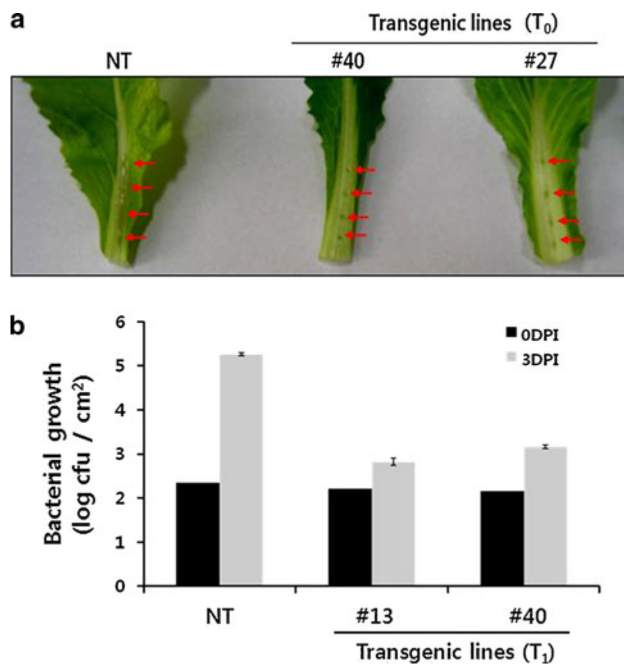

Fig. 3 Disease severity against *Pcc* in transgenic lines. **a** Wounds were generated on the midribs of the leaves of transgenic plants (T_0), and then 5 μl bacterial suspension were dropped on wound. The photo was taken at 1 day after the inoculation, and *arrows* indicate the position of inoculation. **b** Transgenic plants were inoculated with *Pcc* by infiltration. Bacterial numbers were counted at the indicated times

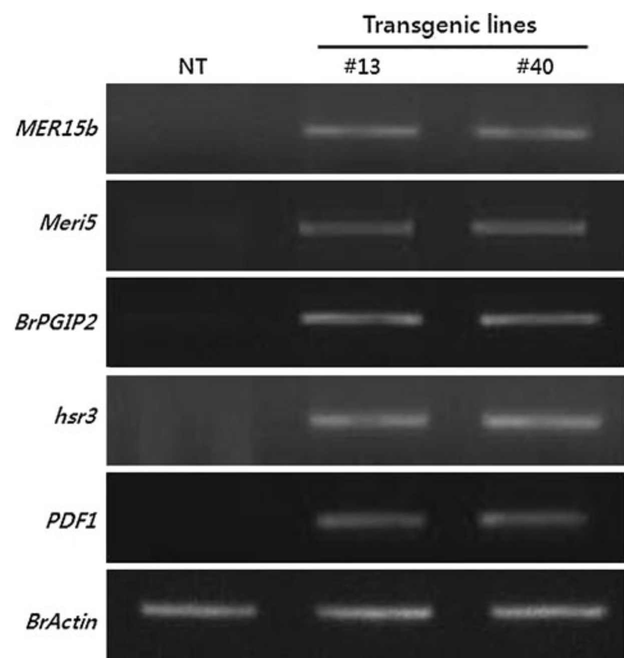

Fig. 4 Expression analysis of defense marker genes in *OsLRP* transgenic plants. RT-PCR was carried out using *OsLRP* transgenic plants and non-transgenic controls. Specific primers were used for two glucanases (*MERI5B* and *Meri-5*), *BrPGIP2*, *PDF1*, *Athsr1* shown in Table 1. *BrActin* is used as a control for the same amount of transcript in each sample

enhanced resistance to bacterial soft rot. To further confirm whether transgenic plants over-expressing *OsLRP* shows resistance to bacterial soft rot, we challenged T_1 transgenic plants with *Pcc* (10^3 cells/ml) by infiltration. Samples were taken at 0 and 3 dpi and bacterial numbers were counted. The black bar in Fig. 3b shows the number of bacterial cells in the samples taken at 0 dpi, while the gray bar shows the number of bacterial cells in the samples taken at 3 dpi. The number of bacteria in the transgenic lines over-expressing *OsLRP* was reduced approximately 20-fold compared to non-transgenic controls, indicating that *Os-LRP* transgenic T_1 plants showed enhanced resistance to bacterial soft rot, as did T_0 plants. Taken together, *OsLRP* conferred reduction of the bacterial growth and symptom of bacterial soft rot.

OsLRP activates defense response in Chinese cabbage

Chinese cabbage over-expressing *OsLRP* exhibits enhanced resistance to *Pcc*. To determine whether *OsLRP* activates defense response in Chinese cabbage, we carried out RT-PCR for defense-associated genes (Fig. 4). We obtained defense marker genes from *B. rapa* ssp. *pekinensis* from an EST collection of the *B. rapa* genomics team in our institute. Two glucanase genes were induced by *Pcc* (Kim and Hwang, unpublished results). As shown in Fig. 4, two different glucanase genes (*MERI5B* and *Meri-5*) and *PDF1* were up-regulated in *OsLRP* transgenic lines #13 and #40. In addition, *BrPGIP2* was also constitutively expressed in *OsLRP* transgenic lines (Fig. 4). It is previously known that *BrPGIP2* is induced upon *Pcc* infection. Moreover, over-expression of *BrPGIP2* exhibits enhanced resistance to bacterial soft rot (Hwang et al. 2010). These results proposed that *OsLRP* activates defense response in Chinese cabbage and confer enhanced resistance to bacterial soft rot. LeLRP and SLRR had been implicated in defense signaling (Tornero et al. 1996; Hipskind et al. 1996). It has been suggested that NtLRP and CaLRR1 are modulators of HIR1-mediated cell death. It has also been suggested that the OsLRR1 and OsHIR1 systems are well conserved in rice (Zhou et al. 2009). Heterologous expression of *OsLRR1* in *Arabidopsis* was shown to confer enhanced resistance to *P. syringae* and activate defense responses (Zhou et al. 2009). Cell death is promoted in transgenic lines over-expressing *OsLRR1*. The authors proposed that OsLRR1 is also modulator of cell death (Zhou et al. 2009). Hence, we also checked whether cell death was promoted in transgenic Chinese cabbage over-expressing *OsLRP*. *Athsr* genes are known to be marker of hypersensitive cell death (Lacomme and Dominique 1999; Pandey et al. 2005). *Athsr3* was constitutively expressed in transgenic Chinese cabbage over-expressing *OsLRP*,

indicating that *OsLRP* also functions as a modulator of cell death in Chinese cabbage. According to the phylogenetic tree of LRR homologs in various plant species, *Os*LRP is very close to *At*LRR1. We also found a *Brassica* homolog of LRR1 through a search of the National Center for Biotechnology Information (NCBI) database. OsLRP showed a 44 % similarity with putative *Br*LRR1 (GenBank accession number EU424347.1). Even though there are no reports on the *Br*LRR-*Br*HIR system, it appears to be conserved in Chinese cabbage. Heterologous expression of *OsLRP* enhances resistance to pathogens in Chinese cabbage and *Arabidopsis*, perhaps due to cell death regulation that results from the activation of defense response. To demonstrate whether the *Br*LRR1-*Br*HIR system is conserved in Chinese cabbage, study is required.

Bacterial soft rot caused by *Pcc* is a major disease of Chinese cabbage. Some genes that show enhanced resistance to *Pcc* have been previously reported (Liau et al. 2003; Jung et al. 2008; Vanjildorj et al. 2009; Ban et al. 2009; Hwang et al. 2010). For example, over-expression of the BAA1 gene (encoding bromelain) from pineapple was reported to show resistance to *Pcc* in Chinese cabbage (Jung et al. 2008). Another example is AiiA encoding *N*-acyl homoserine lactone-lactonase from *Bacillus* sp. GH02. Over-expression of AiiA showed enhanced resistance to *Pcc* (Vanjildorj et al. 2009). This is the first report that LRR homologs show enhanced resistance to the necrotrophic bacterial pathogen, *Pcc*. LRR homologs will be candidates for engineering plants with disease resistance.

Acknowledgments This work was supported in part by two Grants (PJ007850 and PJ008574) from Rural Development Administration (RDA) to Duk-Ju Hwang.

References

Ban H, Chai X, Lin Y, Zhou Y, Peng D, Zhou Yi, Zou Y, Yu Z, Sun M (2009) Transgenic *Amorphophallus konjac* expressing synthesized acyl-homoserine lactonase (aiiA) gene exhibit enhanced resistance to soft rot disease. Plant Cell Rep 28:1847–1855

Hipskind JD, Nicholson RL, Goldsbrough PB (1996) Isolation of a cDNA encoding a novelleucine-rich repeat motif from Sorghum bicolor inoculated with fungi. Mol Plant Microbe Interact 9(9):819–825

Hwang SH, Lee IA, Yie SW, Hwang DJ (2008) Identification of an OsPR10a promoter region responsive to salicylic acid. Planta 227(5):1141–1150

Hwang BH, Bae H, Lim HS, Kim K, Kim S, Im MH, Park BS, Kim D, Kim J (2010) Overexpression of polygalacturonase-inhibiting protein 2 (*PGIP2*) of Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) increased resistance to the bacterial pathogen *Pectobacterium carotovorum* ssp. *carotovorum*. Plant Cell Tiss Organ Cult 103:293–305

Hwang SH, Yie SW, Hwang DJ (2011) Heterologous expression of OsWRKY6 gene in Arabidopsis activates the expression of defense related genes and enhances resistance to pathogens. Plant Sci 181:316–323

Jacques A, Ghannam A, Erhardt M, de Ruffray P, Baillieul F, Kauffmann S (2006) NtLRP1, a tobacco leucine-rich repeat gene with a possible role as a modulator of the hypersensitive response. MPMI 19(7):747–757

Ji GH, Wei LF, He YQ, Wu YP, Bai XH (2008) Biological control of rice bacterial blight by *Lysobacter antibioticus* strain 13–1. Biol Control 45(3):286–296

Jung HW, Hwang BK (2007) The leucine-rich repeat (LRR) protein, CaLRR1, interacts with the hypersensitive induced reaction (HIR) protein, CaHIR1, and suppresses cell death induced by the CaHIR1 protein. Mol Plant Pathol 8(4):503–514

Jung EH, Jung HW, Lee SC, Han SW, Heu S, Hwang BK (2004) Identification of a novel pathogen-induced gene encoding a leucine-rich repeat protein expressed in phloem cells of *Capsicum annuum*. Biochim Biophys Acta 1676(3):211–222

Jung YJ, Choi CS, Park JH, Kang HW, Choi JE, Nou IS, Lee SY (2008) Overexpression of the pineapple fruit bromelain gene (BAA) in transgenic Chinese cabbage (*Brassica rapa*) results in enhanced resistance to bacterial soft rot. Electron J Biotechnol 11:1–8

Lacomme C, Dominique R (1999) Identification of new early markers of the hypersensitive response in Arabidopsis thaliana. FEBS 459(1999):149–153

Lam E, Kato N, Lawton M (2001) Programmed cell death, mitochondria and the plant hypersensitive response. Nature 411:848–853

Lee IA (2005) Isolation and characterization of auxin-regulated gene (BcAXR1) from Chinese cabbage (*Brassica campestris*). Unpublished thesis (M.A.), The Catholic University of Korea

Liau CH, Lu JC, Prasad V, Hsiao H, You SJ, Lee J, Yang NS, Huang HE, Feng TY, Chen WH, Chan MT (2003) The sweet pepper ferredoxin-like protein (pflp) conferred resistance against soft rot disease in *Oncidium orchid*. Transgenic Res 12:329–336

Pandey AK, Ger MJ, Huang HE, Yip MK, Zeng J, Feng TY (2005) Expression of the hypersensitive response-assisting protein in *Arabidopsis* results in harpin-dependent hypersensitive cell death in response to *Erwinia carotovora*. Plant Mol Biol 59:771–780

Ren JP, Petzoldt R, Dickson MH (2001) Screening and identification of resistance to bacterial soft rot in *Brassica rapa*. Euphytica 118:271–280

Tornero P, Mayda E, Gómez MD, Cañas L, Conejero V, Vera P (1996) Characterization of LRP, a leucine-rich repeat (LRR) protein from tomato plants that is processed during pathogenesis. Plant J 10(2):315–330

van der Hoorn RA, Wulff BB, Rivas S, Durrant MC, van der Ploeg A, de Wit PJ, Jones JD (2005) Structure–function analysis of Cf-9, a receptor-like protein with extracytoplasmic leucine-rich repeats. Plant Cell 17(3):1000–1015

Vanjildorj E, Song SY, Yang ZH, Choi JE, Noh YS, Park S, Lim WJ, Cho KM, Yun HD, Lim YP (2009) Enhancement of tolerance to soft rot disease in the transgenic Chinese cabbage (*Brassica rapa* L. ssp. Pekinensis) inbred line Kenshin. Plant Cell Rep 28:1581–1591

Vlot AC, Klessig DF, Park SW (2008) Systemic acquired resistance: the elusive signal(s). Curr Opin Plant Biol 11:436–442

Zhou L, Cheung MY, Zhang Q, Lei CL, Zhang SH, Sun SS, Lam HM (2009) A novel simple extracellular leucine-rich repeat (eLRR) domain protein from rice (OsLRR1) enters the endosomal pathway and interacts with the hypersensitive-induced reaction protein 1 (OsHIR1). Plant Cell Environ 32(12):1804–1820

Zhou L, Cheung MY, Li MW, Fu Y, Sun Z, Sun SM, Lam HM (2010) Rice hypersensitive induced reaction protein 1 (OsHIR1) associates with plasma membrane and triggers hypersensitive cell death. BMC Plant Biol 10:290–299