A Thesis for the Degree of Master of Science

Identification of Target Proteins Required for Cell Death Induced by *Phytophthora infestans* Core Effectors Recognized by Nonhost Pepper

비기주 식물인 고추에서 저항성 반응을 유도하는 감자역병균 core effector 들의 Target 단백질 동정

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Abstracts

Phytophthora infestans is one of the most devastating plant pathogenic oomycetes, which causes severe yield loss of *Solanum* species including economically important crops, such as potato and tomato. Like most successful pathogens, *P. infestans* secretes diverse sets of effector proteins into host cell to promote pathogen proliferation. Contrary, various plant resistance (R) genes recognize effectors and induce effector-triggered immunity (ETI) to suppress pathogen proliferation. Non-host resistance (NHR), the most common form of immune response of plants, is defined as the resistance of a plant species against all isolates of a pathogen species and durable in nature. In recent researches, effectors are regarded as a key factors for understanding host range and also NHR in terms of ETI. Since pepper is non-host plant against *P. infestans*, 57 RXLR effectors were predicted *in silico* using genome information. And these CEs (Core effectors) were transiently expressed in two pepper accessions to identify candidate AVR (avirulence) genes which associated with NHR. Eight CEs induced cell death on CM334 or ECW30R suggesting that these effectors might be recognized by corresponding R genes of pepper. Among them, CE31, CE66 and CE86 (PITG_07550, PITG_15930, and PITG_23226, respectively) were selected for further investigation on their roles in cell death of identifying target proteins. These three putative effectors affected pathogen

growth on host plant. And among them, especially with CE86, heat shock proteins (HSPs) 70kDa homologs were identified as potential host target proteins in host plant, *Nicotiana benthamiana*. Furthermore, exploiting CE31 and CE66 to identify NHR genes which recognize these two effectors by co-expressing with 415 pepper NB-LRR coding genes, but none of these putative R genes induced cell death when co-expressed with selected CEs. In conclusion, screening results from this paper suggest the multiple candidate effectors which possibly involved in NHR of pepper against *P. infestans*. And three selected effectors (CE31, CE66, and CE86) could be exploited for further experiments to understand more detailed mechanisms of NHR in terms of ETI.

Key words: Potato late blight pathogen (*Phytophthora infestans*), Effectors, Non-host resistance (NHR), Effector-triggered immunity (ETI), Target proteins

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LIST OF ABBREVIATIONS

Avr	Avirulence
Co-IP	Co-immunoprecipitation
ETI	Effector-triggered immunity
HR	Hypersensitive response
LC-MS	Liquid chromatography mass spectrometry
MAPK	Mitogen-activated protein kinase
NHR	Non-host resistance
NLR	Nucleotide-binding leucine-rich repeat
PAMP	Pattern-associated molecular patterns
PCD	Programmed cell death
Pex	Phytophthora extracellular
PRR	Pattern recognition receptors
PTI	PAMP-triggered immunity
PVX	*Potato virus* X
R	Resistance
RT-PCR	Reverse transcription polymerase chain reaction

INTRODUCTION

Phytophthora infestans, the causal agent of potato late blight, is one of the most devastating plant pathogenic oomycetes. According to previous reports, the costs to control the potato late blight reaches to $ 6.7 billion per year (Haverkort *et al*., 2008). The difficulty of the controlling late blight disease and its severe pathogenic potential stimulated to find various strategies for resistance breeding. And there have been lots of approaches to breed resistance potato cultivars by exploiting plant innate immune system.

Plants have evolved two-layered defense systems that are activated in response to pathogen invasions (Dangl *et al.,* 2013). Plant recognize universally conserved components of pathogenic microorganisms, known as pathogen-associated molecular patterns (PAMPs) by membrane-bound receptor proteins called pattern recognition receptors (PRRs), and induce PAMP-triggered immunity (PTI) to halt pathogen proliferation. (Dodds *et al.*, 2010). Meanwhile, successful pathogens are able to suppress basal defense mechanisms of plant by secretion of effector proteins into plant cell. Effectors interact with host target proteins or genes and consequently modulate host physiology or promote infection, leading to effector-triggered susceptibility (ETS) (Jones and Dangl, 2006; Kamoun, 2006). And during evolution, plant have possess hundreds of nucleotide binding domain leucine-rich repeat (NLR) resistance proteins that recognize effectors and induce effector-triggered immunity (ETI) (Dodds and Rathjen, 2010; Flor, 1971). Typically, ETI elicit hypersensitive response (HR), a localized programmed cell death (PCD). Several NLR genes of wild *Solanum* species have been identified as

resistance (R) genes against *P. infestans*. For example, eleven R genes (*R1 – R11*) from *S. demissum*, and two R genes (*Rpi-blb 1* and *Rpi-blb 2*) from *S. bulbocastanum*, were introgressed into commercial potato cultivars for improving resistance cultivars against *P. infestans* (Vleeshouwers *et al*., 2011.). However, rapid occurrence of highly virulent *P. infestans* isolates results in the breakdown of these single R gene-mediated resistance. In a coevolutionary arms race with resistant potato cultivars, *P. infestans* evades R-gene mediated recognition via mutation or changes expression level of the genes encoding corresponding effectors (Anderson *et al*., 2015; Fry *et al*., 2008).

Phytophthora species secrete and translocate a number of effectors into host cells (Birch *et al*., 2008). The RXLR motif required for targeting host cells is a common feature of identified avirulence effectors such as ATR1, Avr3a, Avr1b, and Ipi-O in diverse oomycetes (Rehmany *et al*., 2005; Whisson *et al*., 2007). Therefore, hundreds of putative RXLR effector genes have been predicted also in *Phytophthora* genome, such as 563 RXLR effector coding genes in *P. infestans* (Haas *et al*., 2009). Furthermore, catalogue of genome-based predicted effectors enables high-throughput screening of effector activity called effector-omics (Anderson *et al*., 2015; Vleeshouwers *et al*., 2011.). For example, suppression of PCD by 169 RXLR effectors of *P. sojae* was screened and 107 effectors consistently suppressed BAX-induced cell death (Wang *et al*., 2011). In addition, 64 RXLR effectors of *Hyalaperonosopora arabidopsis* were transformed into *Pseudomonas syringae* and screened by observing effector-mediated effects on bacterial growth in host and non-host plants (Fabro *et al*., 2011).

Recently, functional studies about RXLR effectors generally carried out in two perspectives. First, identification of *in planta* target proteins for understanding detailed molecular mechanisms of effectors. As previously mentioned, effectors generally modulate plant physiology, but only a few oomycetes effectors contain predictable functional domains or motifs. Therefore, identification of in planta target proteins is important to investigate effectors molecular mechanisms. Several host targets of *P. infestans* RXLR effectors have been reported. Avr3a targets and stabilizes E3 ligase CMPG1 and suppress defense-related cell death response during infection (Bos *et al*., 2010). Avrblb2 targets cysteine protease C14 and prevents secretion of this defense-related protease (Bozkurt *et al*., 2011). Second, identification of durable resistance genes corresponding to effectors in terms of ETI. As previously mentioned, single R-gene-mediated resistance of potato against *P. infestans* could be easily broken. Therefore, lots of efforts have been put into establishing more durable strategy, and non-host resistance (NHR) is one possible method for durable resistance against *P. infestans* (Heath, 1985).

NHR is resistance shown by an entire plant species against to a majority of potential pathogenic microorganisms which governed by multiple genetic factors while host resistance is governed by single major resistance gene and thereby easily broken (Heath, 2000). NHR responses against *P. infestans* in various plants species are characterized with highly localized cell death-associated defense also known as hypersensitive response (HR) (Huitema *et al*., 2003; Vleeshouwers, *et al*., 2000.), and another research suggest that multiple RXLR effector recognition is associated with NHR of *Capsicum annuum* against *P. infestans* in terms of ETI (Lee *et al*., 2014). Indeed, several R genes have been reported to be

involved in NHR. In Poaceae, Rxo1 was introduced from maize to rice and conferred resistance to bacterial speck disease caused by *Xanthomonas oryzae,* and likewise WRR4 was transferred between Brassicaceae plants from Arabidopsis to *B. napus* and conferred resistance to incompatible oomycetes pathogen, *Albugo candida* (Borhan *et al*., 2010; Zhou *et al*., 2010).

In this study, non-host interaction of pepper against *P. infestans* was investigated using effector-omics approach. Therefore, 57 RXLR effectors challenged on two accessions of non-host pepper to identify candidate AVR genes. Based on screening results, several candidates were selected and confirmed whether putative effectors could affect pathogen growth as an effector. *In planta* co-expression assay was performed for identifying pepper R genes which recognizing screened *P. infestans* CEs. And furthermore, host target proteins of selected CEs were identified by *in planta* immunoprecipitation (IP) and LC-MS/MS assay to investigate detailed molecular mechanisms of selected effectors.

LITERATURE REVIEWS

The Potato Late Blight Pathogen (*Phytophthora infestans*)

P. infestans causes the Potato late blight, one of the most notorious diseases in *Solanum* crops such as potato and tomato. Since this pathogen has been a major cause of historic starvation called the Irish famine in Europe in 1845, it still threatens the food-security so far. *P. infestans* is a heterothallic oomycetes, which is distinguishable from fungi and rather similar to the golden-brown algae (Latijnhouwers *et al.,* 2003), and is a hemi-biotrophic pathogen under natural or agricultural conditions (Fry, 2008). The characteristics of *P. infestans* life cycle are closely related to its serious pathogenicity. *P. infestans* produces dispersive sporangia containing motile bi-flagellated zoospores during asexual life cycle, which enable widely spread and continuous infections. Even during initial infection stage of 4 days, no signs are observed, making it difficult for farmers to take appropriate action for prevention. After this period, hyphae grow out from the infected tissue accompanied by necrotic cell death. The life cycle described has a poly-cyclic characteristic that repeats continuously over a period of 5 days under appropriate conditions (Judelson & Blanco, 2005).

Severe pathogenic potential of *P. infestans* stimulated various strategies to prevent potato late blight. Although chemical control using fungicides is regarded as a major method, has difficulty with the timing of treatment. Therefore, lots of efforts have been focused on breeding resistant cultivars using the plant resistance mechanisms. Various tuber-bearing wild *Solanum* species from central Mexico and

Andean region, where *P. infestans* are also originated show resistance against *P. infestans* (Carbone & Ristaino, 2007). These resistance loci were introduced into commercial potato cultivars for durable resistance and some of them have been cloned. Several R genes against *P. infestans* belong to NLR (nucleotide binding leucine rich repeat) gene family and recognize corresponding effectors, such as *R1, R3a* from *S. demissum*, and Rpi-blb family genes from *S. bulbocastanum* (Vleeshouwers *et al*., 2011). However, resistance mediated by those R genes are easily broken due to rapid mutation or gene expression changes of *P. infestans* effectors, which enable them to overcome resistance induced by a single R genes. (Anderson *et al*., 2015; Fry *et al*., 2008).

Effectors

A large number of proteins secreted by pathogens, called effectors, are translocated into plant cells to modulate host cell structure or function. (Anderson *et al*., 2015) Effectors are classified into two classes, apoplastic and cytoplasmic effectors. Apoplastic effectors secreted into plant extracellular space interact with extracellular target or are recognized by membrane-bound receptors. In contrast, cytoplasmic effectors are translocated into cytoplasm or nuclear region and interact with cytological target or recognized by cytoplasmic disease-resistance proteins called NLR (Belkhadir *et al*., 2004; Kamoun, 2006). Effectors generally facilitate pathogen infection by perturbing plant defense mechanisms via interacting with specific target proteins. On the other hand, effectors could be recognized by plant surveillance systems and consequently trigger defense response as elicitors, suggesting these proteins affect positively or negatively on the outcome of

pathogen infection, and these results are determined by the repertoires of pathogen avirulence factors (effectors) and plant resistance receptors. Therefore, identification and research of effectors is worthwhile to prevent disease which caused by plant pathogenic microorganisms. (Anderson *et al*., 2015; Kamoun, 2006)

Effector proteins must be secreted and delivered in order to reach their cellular targets (Boevink *et al*., 2016). Therefore, effectors contain specific amino acid sequences called signal peptides for secretion or translocation. Recently, a large set of effectors have been predicted bio-informatically on the oomycetes genomes based on structural characteristics of effectors. For example, Pexs (*Phytophthora* extracellular proteins) were identified from *Phytophthora* ESTs (Expressed sequence tags) according to whether it contain a putative signaling peptide for secretion (Torto *et al*., 2003). Furthermore, RXLR (Arg – X – Leu – Arg) motif which is similar to signal peptides required for translocation first discovered from malaria parasites (*Plasmodium* spp.) is known to be conserved in various oomycetes effectors (Rehmany *et al*., 2005), and this has also been shown to be involved in the delivery of effectors into host cell (Whisson *et al*., 2007). Similarly, RXLR motif also served as an important feature for bioinformatic screening of effectors. Especially 563 RXLR effectors predicted in *P. infestans* genome (Haas *et al*., 2009).

Genome-wide identification of hundreds of potential RXLR effectors stimulates functional genomics researches which performed to investigate function and detailed mechanisms of predicted effectors in a large scale (Anderson *et al*., 2015). For example, Oh *et al*. identified Avrblb2 effector which is recognized by *Solanum bulbocastanum* NLR, *Rpi-blb2* by *in planta* co-expressing 62 RXLR

effectors of *P. infestans* with *Rpi-blb2* in *N. benthamiana* (Oh *et al*., 2009). Similarly, 169 *P. sojae* RXLR effectors were transiently expressed in *N. benthamiana* to investigate whether these genes could suppress cell death induced by oomycetes and mammalian cell death elicitor INF1 and Bax, respectively. Most of *P. sojae* RXLR effectors could suppress PCD triggered by elicitors suggesting potential of tested RXLR effectors to suppress PCD-mediated plant defense (Wang *et al*., 2011). Furthermore, Fabro *et al*. exploited effector-detector vector (EDV) system which enables secreting oomycetes RXLR effectors through the pseudomonas TTSS (type-three secretion system) to investigate function of single effector during compatible and incompatible interactions by monitoring bacterial growth (Fabro *et al*., 2011; Sohn *et al*., 2007). These series of results suggest that various effectors play a role in determining the resistance or susceptibility between plant and pathogen interactions.

On the other hand, host target protein identifications have been performed to investigate detailed molecular function of effectors. Structure-based predictions of effector function have not been successful because among a number of effectors very few of them possess functionally recognizable motifs or domains, such as bacterial effector AvrPtoB and Avr3b of *P. sojae* (Dong *et al*., 2011; Jonas *et al*., 2006). To date, various types of molecular components have been identified as the targets of effectors. For example, *P. infestans* Avrblb2 targets C14 protease and prevents secretion of it which positively function for immune response against *P. infestans* (Bozkurt *et al*., 2011). Likewise, *P. infestans* Avr3a targets and stabilizes host U-box E3 ligase protein CMPG1, which is degraded during INF1-mediated cell death (ICD) response in natural condition, for suppressing defense related

ICD (Bos *et al*., 2010). *P. sojae* CRN (crinkling- and necrosis- inducing proteins) effectors PsCRN63 and PsCRN115, both effectors interact with *N. benthamiana* catalase and jointly manipulates plant PCD by perturbing H_2O_2 homeostasis of plant (Liu *et al*., 2011; Zhang *et al*., 2015). And furthermore, heat shock protein 70 (HSP70) which protein function as molecular chaperones is targeted by bacterial effector HopI1 and resulted in enhancing susceptibility in plants (Jelenska *et al*., 2010). In contrast, pepper CaHSP70 reported as protein target of *Xanthomonas* type III effector AvrBsT which suppresses pathogen growth with PCD by directly associating with AvrBsT (Kim *et al*., 2015).

In conclusion, effectors target various array of pathways generally to increase the fitness of the pathogen, and in some cases these interaction could negatively function for pathogenicity. Studies of the molecular mechanisms of effectors can also be useful to confer resistance to crops against pathogens. (Bozkurt *et al*., 2011).

Resistance genes

Resistance response of plants against pathogens is correlated with genetic interactions between specific plant R gene and pathogen Avr gene called gene for gene resistance (Flor, 1971). Since the first R genes, *RPS2*, and *N* cloned from Arabidopsis and tobacco, respectively, numerous R genes have been cloned from a various plant species (Lee & Yeom, 2015; Mindrinos, 1994; Whitham, 1994). The majority of resistance genes encode cytoplasmic receptor-like proteins which contain nucleotide-binding (NB) site and leucine-rich repeat (LRR) domain called NLR proteins (Baker *et al.,* 1997). NLR proteins trigger innate immune response

called effector-triggered immunity (ETI) by recognizing pathogen effectors directly or indirectly (Qi & Innes, 2013). Typically, ETI induced pathogen growth inhibition accompanied with hypersensitive response (HR), a localized programmed cell death (PCD), however HR and resistance can be uncoupled in some cases (Coll *et al.,* 2011).

Among the identified R and Avr gene pairs, direct interactions were observed only in several cases. For example, direct interactions between flax resistance genes L5 and L6, and variants of flax rust pathogen (*Melampsora lini*) effector AvrL567 were confirmed by yeast-two hybrid (Dodds *et al*., 2006; Ravensdale *et al*., 2012). *Rpi-blb1* from *S. bulbocastanum*, and *RPP1* from Arabidopsis were also reported as directly interact with corresponding effectors (Chen *et al*., 2012; Krasileva *et al.,* 2010). In contrast, other NLRs indirectly recognize effectors, in terms of guard model that plant NLRs activated by monitoring (guarding) the other host proteins (guardee) of their corresponding effectors (Hoorn *et al.,* 2008; Mchale *et al*., 2006). Interaction between protein kinase Pto, and R protein Prf and bacterial effector, AvrPto/AvrPtoB is well-known case of indirect interaction. Immunity in tomato to *P. syringae* expressing AvrPto or AvrPtoB requires both Pto and Prf (Mucyn *et al*., 2006). Interestingly, structure of Pto is resembles to cytoplasmic kinase domains of FLS2 and EFR which kinases are function for plant defense and targeted by AvrPto and AvrPtoB. And in association with R protein Prf, Pto function as guardee of AvrPto/AvrPtoB by direct association, and trigger resistance (Xiang *et al.,* 2008). In Arabidopsis, RIN4 that works with RPM1 and RPS2 was also reported as a guardee protein (Mackey *et al*., 2002, 2003). Likewise, indirect effector recognition allows resistance to

multiple effectors even with limited repertories of R gene (Hoorn & Kamoun, 2008). Furthermore, as the structure and function of NLRs have been studied in detail, cases of NLR pairing have been reported. Several NLRs require another NLR to confer resistance, such as Arabidopsis RRS1 and RPS4, rice RGA4 and RGA5 (Cesari *et al.,* 2013; Narusaka *et al*., 2009). In conclusion, ETI of plants occurs in a complex manner and further studies of NLR mechanisms will be needed.

Non-host Resistance

Plants are exposed to numerous plant pathogens. However, most of plants are resistance to most of potential pathogens. These immune response shown by an entire plant species against all genetic variants of pathogen species is called non-host resistance (Heath, 2000). NHR is known to be the most common and durable form of resistance, and therefore NHR could be exploited for durable disease management (Kamoun, 2001).

Molecular mechanisms of NHR have been studied at multiple level of plant defense systems from constitutive barriers to inducible reactions. It has been reported that various factors of host resistance are also involved in NHR (Schulze-lefert & Panstruga, 2011; H. A. Lee *et al.,* 2017). For example, Lipka *et al*. reported that resistance response of *Arabidopsis* against non-adapted pathogen *Blumeria graminis* f. sp. *hordei* is compromised in Arabidopsis mutants impaired in pre- or post- invasive defense (Lipka *et al*., 2005). Likewise, mutation of defense signaling cascade genes of *Arabidopsis* (Brassiccaceae) or *N. benthamiana* (Solanaceae) support that PTI (*BAK1*, and *BIK1*) and ETI (*EDS1*, *SGT1*, *HSP70*,

HSP90, and *SIPK*) related components also contribute to NHR (Heese *et al*., 2007; Kanzaki *et al*., 2003; Kemmerling *et al*., 2007; Moreau *et al*., 2012; Peart *et al*., 2002; Roux *et al*., 2011; Sharma *et al*., 2003).

Furthermore, several functional PRRs or NLRs have been identified from nonhost plants. ELR (elicitin response), which recognize *P. infestans* PAMP INF1, was identified from *Solanum microdontum*. And transfer of ELR into commercially cultivated potato resulted in enhanced resistance to *P. infestans* (Du *et al*., 2015). Similarly, *Rxo1*, a maize NLR gene, which recognize AvrRxo1 of non-adapted *Xanthomonas oryzae* pv. *oryzicola* was transferred into another Poaceae species, rice (*Oryzae sativa* cv. *Kitake*) and conferred resistance against *X. oryzae* pv. *oryzicola* (Zhao *et al*., 2004; Zhao *et al*., 2005). And even, transfer of the *Arabidopsis* NLR coding genes *RPS4* and *RRS1* into solanaceae crop tomato conferred resistance against bacterial pathogen *Ralstonia solanacearum* (Narusaka *et al*., 2013). These results imply that the R gene-mediated downstream signaling cascades could be highly conserved through in related species. And therefore, functional inter-species or family transfer of nonhost R genes could be a useful strategy for providing durable and powerful resistance. (Lee *et al.,* 2017)

Materials and Methods

Plant materials and growth conditions

Seeds of *Nicotiana benthamiana* were sowed and grown in the 23~25^{o}C chamber with 60% relative humidity and a 16 h: 8 h light and dark period time ratio were maintained. Two-week-old seedlings were transplanted into separated pot. Seeds of peppers (CM334 and ECW30R) were disinfected with 2.5% sodium hypochlorite (NaOCl) and germinated in 30^{o}C with 100% relative humidity condition. After germination, pepper seedlings were transplanted 200-plug form tray and grown for 2 weeks in same condition such as *N. benthamiana* grown. Two-weeks-old pepper seedlings were transplanted into 50-flug form tray. Commercial horticultural soil media were used for both tobacco and pepper plants (Biogreen,).

Construction of plasmid vectors

57 CEs were provided from Dr. Sophien Kamoun (The Sainsbury Laboratory, Norwich, UK.). All CEs were cloned into *potato virus X* (PVX) based vector pICH31160 (pkw) and transferred to *Agrobacterium tumefaciens* strain GV3101 (Seo., 2015). Especially, 8 CEs (CE31, 34, 47, 49, 66, 70, 82, and 86) were also cloned into pCAMBIA2300 (pc2300) non-viral binary vector to exclude virus effects during *in planta* transiently expression. Cell death phenotypes of pc2300::CEs were significantly compromised compared to pkw::CEs but still remained, and similar changes were observed in expression level.

Effector screening on non-host pepper

Agrobacterium carrying core effector genes in *potato virus X* (PVX) vector pkw were incubated in YEP liquid media for a day. Cultured medium were centrifuged at 3,000 rpm for 8 min to spin down all suspended cells, and then cell pellet was resuspended into 1ml of infiltration buffer (10 mM MES, 10 mM $MgCl_2$, and 150 μM acetosyringone), and diluted to final OD600 values for 0.4. For an efficient transformation, the suspension was incubated with 200 rpm shaking for an hour, then infiltrated into expended leaves of 4~5 week-old *N. benthamiana* by using needless syringe. At 2 dpi (day post inoculation), infiltrated leaves were ground for a virus inoculum in liquid nitrogen. Virus inoculum was suspended in 500 μl of 0.05M K_2HPO_4 buffer, and rubbed onto the first normal leaves of 4~5 week-old pepper (CM334 and ECW30R) after mixed with 400-mesh carborundum. After they were completely dried out, carborundum was washed out by using distilled water. At 6~8 dpi, inoculated leaves were harvested and cell death phenotypes were observed. Leaf chlorophyll was eliminated with 100% ethanol for 2~3 days at 60^{o}C.

Virulence test

Agrobacterium-mediated transiently over-expression was performed on *N. benthamiana* leaves and the leaves were detached at 1 dpi (left-half: pc2300::EGFP; right-half: pc2300::candidate effectors). *P. infestans* zoospores ($1.0x10^5$ spores/ml) were inoculated on these leaves. Inoculated leaves were incubated at the room temperature of 23~25^{o}C with 100% relative humidity condition. Depends on

development of lesions, lesion size were measured at 4~6 dpi (days post inoculation) using Image J program

In planta immunoprecipitation (IP) assay

Protocol described in Saunders *et al* was modified (Saunders *et al*. 2012) and performed as described in Lee *et al* (Lee *et al*., unpublished). Three epitope FLAG tag were attached in front of CE86 coding region. 3xFLAG::CE86 was transiently expressed in *N. benthamiana* by agro-infiltration, 3xFLAG::GFP exploited as a negative control. Two days after, total proteins were extracted and 10mg of total protein extract was incubated in FLAG affinity gel (BioLegend cat.) for 2 hours at 4^{o}C. After three times washing with cold GTEN buffer including 0.1% Tween-20, the precipitated proteins were eluted by boiling with Laemmli sample buffer (Laemmli, 1970).

Virus-induced gene silencing and cell death assay

Agrobacterium containing pTRV1 and pTRV2, carrying partial cDNA of target gene were cultured separately for 12~16 h, and prepared for *in planta* transiently over-expression as previously described. Then, mixed cultures containing pTRV1 and pTRV2 in same ratio and infiltrated on expanded two lower (first & second) leaves of 3~4 week old *N. benthamiana*. After 2 weeks, confirmed successful silencing by observing phenotype of pTRV2-PDS (Phytoene desaturase) plant, and collected several leaf disks for RT (Reverse transcription)-PCR analysis. CE86 were infiltrated on silenced leaves (4~6 leaves apart from pTRV infiltrated leaves). At 4~5 dpi, infiltrated leaves were detached.

RT-PCR assay

RT-PCR was performed for analyzing gene expression of silenced plants. RNA was extracted using TRIZOL reagents (Thermo Fisher Scientific), and cDNA was synthesized from extracted RNA using SuperScript II Reverse Transcriptase (Thermo Fisher Scientific). RT-PCR was performed using specific primer sets which amplifying partial region of silenced genes.

RESULTS

Non-host pepper and host *N. benthamiana* respond differently to *P. infestans* core effectors

Oh *et al*. reported that various pepper accessions showed diverse cell death responses to 54 Pex (*Phytophthora* extracellular protein) RXLR effectors of non-adapted *P. infestans* suggesting that nonhost pepper possess putative receptors recognizing *P. infestans* effectors (Lee *et al*., 2014; Oh *et al*., 2009). Using genome information of four virulent isolates of *P. infestans*, RXLR effectors that are conserved among *P. infestans* and show high level of expression during biotrophic stage were chosen as 57 core effectors (CEs). And to investigate the response of pepper against CEs of nonhost pathogen, we screened CEs-induced cell death on two pepper accessions. According to preliminary screening, 31 of the 57 CEs which exhibited no phenotypes on pepper were excluded from screening candidates.

Transiently expression of 26 CEs was performed on two pepper accessions CM334 and ECW30R using recombinant PVX-virion. This method is optimized for efficient expression in pepper because pepper exhibited very low efficiency to Agrobacterium-mediated transiently expression which are generally used in other plant species including *N. benthamiana* (Lee *et al*., 2014). Among 26 CEs, 13 CEs exhibited HR-like cell death on CM334 including Avrblb2 family (CE17, 19, 73, 74, and 75) which previously reported as HR inducing effectors on CM334 were used for positive control (Lee *et al*., 2014). Of the 13 HR-inducing CEs, except CE34, other CEs also induced cell death on ECW30R (Table 1, Figure

1). These results support that multiple recognition of RXLR effectors is associated with NHR of pepper against *P. infestans* (Lee *et al*., 2014). However, except CE31 and 66, other CEs which induced HR-like cell death on pepper also induced cell death on host plant *N. benthamiana* (Figure 1). Based on effector screening result, CE31 (PITG_07550), CE66 (PITG_15930) and CE86 (PITG_23226) were selected for further phenotypic analysis. CE31 and CE66 were selected because these effectors could be exploited as molecular probes for identifying R genes which associated with NHR of pepper against *P. infestans* by using *in planta* co-expression in *N. benthamiana* system. And CE86 which induced most obvious and stable cell death on both host *N. benthamiana*, and non-host pepper (Figure 1), was selected for investigating detailed molecular mechanisms of its cell death phenotype.

Table 1. *P. infestans* core effectors-mediated cell death response on non-host peppers and host *N. benthamiana*

			C. Annuum		*N. benthamiana*
CE no.	**Effector Id.**	**Annotation**	**CM334**	**ECW30R**	
14	PITG_00582		-	Nd	+
16	PITG_02860		-	Nd	Nd
***17**	PITG_04085	AVRblb2 family	+	+	-
***19**	PITG_04090	AVRblb2 family	+	+	-
31	PITG_07550		+	+	-
32	PITG_07630		-	-	Nd
33	PITG_09160		-	Nd	Nd
34	PITG_09216		+	-	+
35	PITG_09218	PexRD52	-	Nd	+
39	PITG_10540	PexRD5	+	+	+
47	PITG_13044		+	+	+
49	PITG_13048		+	+	+
50	PITG_13093		-	-	-
51	PITG_14371	AVR3a, PexRD7	-	Nd	-
56	PITG_15114		-	-	-
58	PITG_15123		-	-	-
66	PITG_15930		+	+	-
70	PITG_17063	PexRD44	+	+	+
71	PITG_17309		-	Nd	+
72	PITG_17316		-	Nd	+
***73**	PITG_18683	AVRblb2 family	+	+	-
***74**	PITG_20300	AVRblb2 family, PexRD39	+	+	-
***75**	PITG_20301	AVRblb2 family	+	+	-
77	PITG_21362		-	-	+
82	PITG_22804		+	+	+
***86**	PITG_23226		+	+	+
m.cherry			-	-	-

Nd: Non-determined, Avrblb2 family (positive control) marked as asterix (*)

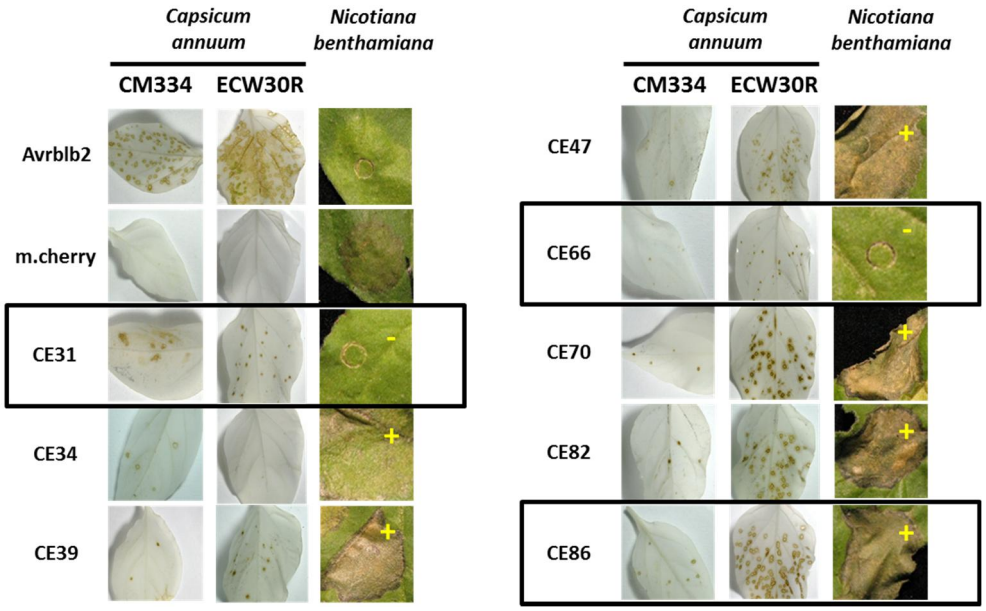


Figure 1. Non-host pepper and host *N. benthamiana* respond differently to nine CEs of *P. infestans*

PVX recombinant virions carrying CEs were transiently expressed on leaves of pepper accessions by mechanical inoculation. Inoculated leaves were detached at 6 dpi and de-stained in 100% EtOH. Besides, CEs were transiently expressed on *N. benthamiana* leaves by agro-infiltration, and infiltrated leaves were detached at 4~5 dpi (Cell death phenotypes marked as '+', and no cell death marked as '-'). Eight CEs induced HR-like cell death on CM334 pepper. CE31, CE66, and CE86 were marked with square boxes.

Cell death phenotypes of CEs on *N. benthamiana* exhibited in a dosage-dependent manner, and were not affected by virus backbone of PVX virus

PVX (*Potato virus X*)-based vector was exploited for CE-mediated cell death screening on pepper and *N. benthamiana*. However virus backbone of pICH31160 vector could affect to CEs-mediated cell death phenotypes on tested plants. Therefore, to exclude viral effects, eight CEs (CE31, 34, 47, 49, 66, 70, 82, and 86) were cloned into pCAMBIA2300 binary vector and infiltrated on *N. benthamiana* leaves by agro-infiltration. Although pCAMBIA2300::*CEs* infiltrated regions exhibited markedly compromised cell death phenotypes including almost disappeared cases of CE70 and CE82, cell death phenotypes were qualitatively maintained (Figure 2a.).

Especially, co-expression of pCAMBIA2300::*CE86* with virus vector control, pICH31160::*m.cherry*, exhibited no differences in cell death phenotype compared to pCAMBIA2300::*CE86* solely infiltration (Figure 2b.). And expression level of CE86 were significantly decreased when contained in pCAMBIA2300 compared to pICH31160 (Figure 2c.). In addition, cell death phenotype of pCAMBIA2300::*CE86* was also retained in ECW30R pepper (Figure 2d). Taken together, these results suggest that cell death phenotypes on tested plants were caused of biological function of each CEs which following dosage-dependent manner, not due to viral effect.

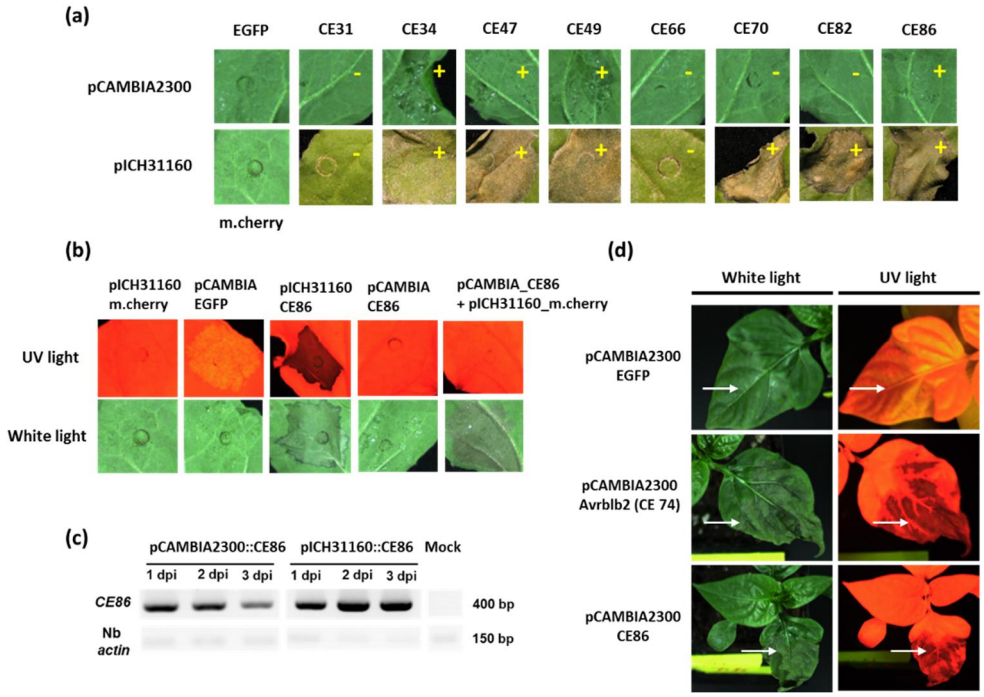


Figure 2. Core effector-induced cell death on *N. benthamiana* was affected by dosage-dependent not by PVX viral vector

(a) Eight CEs were transient expressed on *N. benthamiana* leaves by agro-infiltration. Effector-induced cell death was marked with '+' symbols. (b) *CE86* cloned into both PVX-based pICH31160 and the binary vector pCAMBIA2300 was agro-infiltrated on *N. benthamiana* leaves, photograph were taken at 4 dpi. (c) RT-PCR with cDNA extracted from *N. benthamiana* leaves expressing pCAMBIA2300::*CE86* and pICH31160::*CE86* respectively. (d) Agrobacterium carrying pCAMBIA2300::*CE86* was agro-infiltrated into the leaves of ECW30R. EGFP and Avrblb2 were used as negative and positive control, respectively. Photograph were taken at 4 dpi.

CE31, CE66 and CE86 expression differentially affected *P. infestans* lesion size on *N. benthamiana* leaves

To investigate whether CE31, CE66 and CE86 could function during *P. infestans* infection, pCAMBIA2300::*CE*s were infiltrated on half of *N. benthamiana* leaf, and pCAMBIA2300::*EGFP* infiltrated as a negative control on other half of leaf via agro-infiltration 24 hours prior to *P. infestans* zoospore inoculation. Average lesion size of each inoculation sites measured 4~6 days after zoospores inoculation (4 dpi for CE86 and Avrblb2, and 5~6 dpi for CE31 and 66). According to previous reports, Avrblb2 (CE74, PITG_20300) expression enhances susceptibility of *N. benthamiana* against to *P. infestans* which accompanied with increased lesion size (Bozkurt *et al*., 2011). Thereby *Avrblb2* were selected as a positive control. Expression of *CE74* indeed increased average lesion size of *P. infestans* on *N. benthamiana* leaves compared to lesions from *EGFP* (Figure 3a). Likewise, *CE86* also increased lesion size (Figure 3b), however, in contrast, average lesion sizes from *CE31* or *CE66* expressing region were significantly decreased compared to *EGFP* on *N. benthamiana* leaves (Figure 3c and 3d). Taken together, these result suggest that CE31, CE66 and CE86 play distinct roles in *P. infestans* infection, and could affect pathogen growth.

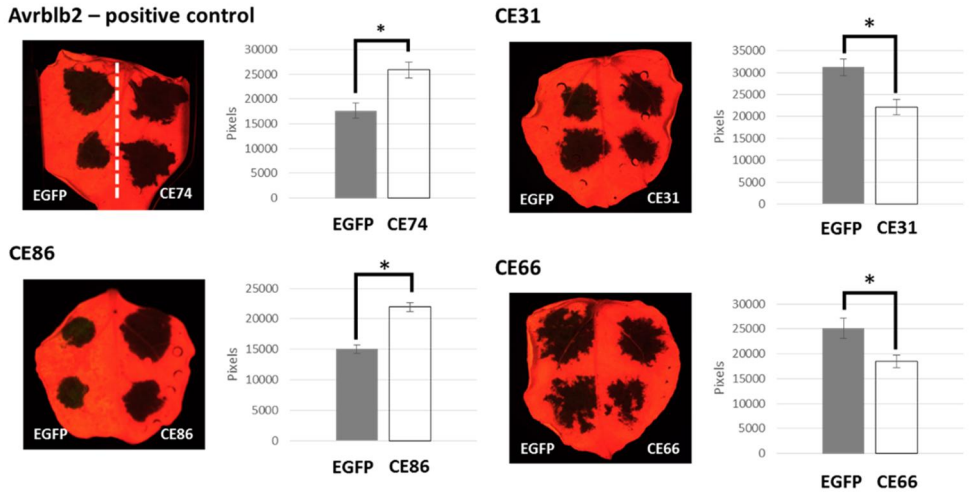


Figure 3. Expression of CE 31, 66 and 86 differentially affected to *P. infestans* growth on *N. benthamiana*

Agrobacterium carrying pCAMBIA2300::*CEs* were infiltrated on half of *N. benthamiana* leaves. The other half of leaf was infiltrated with Agrobacterium carrying EGFP as a negative control. The infiltrated regions were inoculated with *P. infestans* zoospores ($1.0x10^5$ zoospores/ml). Photograph were taken at 4~6 dpi and lesion size were measured using Image J program. One way to Anova test was performed to determine the significant difference of *P. infestans* growth on between EGFP- and CE- infiltration regions. (asterix mark indicate statistical significance, p-value < 0.05)

In planta co-expression of CE31 or CE66 with Non-host pepper NB-LRR genes for identifying NHR genes

CE31 and CE66 are putative *Avr* genes which induce HR-like cell death on non-host pepper. Moreover, these effectors have no phenotypes when transiently expressed by agro-infiltration on *N. benthamiana* (Figure 1). Therefore, co-expression of CE31 and CE66 with NLR coding R genes of pepper (CM334) was performed for identifying NHR genes which recognize these effectors as and induce HR-like cell death as a result of ETI (Oh *et al*., 2009).

Among the 755 NLR genes predicted in pepper (CM334) genome sequence, 289 partial genes were filtered out, and 415 of 466 full-type NLR genes were cloned into pCAMBIA2300 binary vector, and transformed into agrobacterium (Kim *et al*., 2014; Seo *et al.,* 2016). These NLRs were exploited for co-infiltration. Combination of *Rpiblb2* and *Avrblb2* were used as positive control (Oh *et al*., 2009), and pICH31160::*m.cherry* was co-infiltrated with every tested NLRs as a negative control. As a result, none of tested pepper NLRs induced HR cell death in *N. benthamiana* when co-infiltrated with CE31 or CE66 (Table 2). Although several NLRs have yet to be used for experiments, these results suggest that NLRs may need another components to recognize CE31 or CE66.

Table 2. Co-infiltration

Effectors	HR induced / Tested R genes	*Remaining groups
CE31	0 / 366	G2, TNLs
CE66	0 / 306	G1, G2, TNLs

(*NLR classification follows Seo *et al.*, 2016)

NbHSP70 and NbDnaK are putative host targets of *P. infestans* CE86 and play role on plant defense response

CE86 trigger cell death on both host and non-host plants (Figure 1a). Therefore, to investigate cell death mechanisms of CE86 in a molecular level, potential protein targets in *N. benthamiana* were identified using *in planta* immunoprecipitation (Lee *et al.,* unpublished) followed by liquid chromatography-Mass spectrometry analysis (LC-MSMS). As a result, several putative target proteins identified (Table 3). Interestingly, most of identified target proteins were heat shock protein (HSP) family members, including HSP70s (or DnaK), 60 kDa chaperonins homologs, and HSP40-MIP1.1b. Especially, seven NbHSP70 homologs were identified from LC-MS/MS as putative target proteins of CE86. Among them, NbHSP70 (Niben101Scf00117g02019.1) and DnaK (Niben101Scf12868g00008.1) were selected for further studies, because these two genes could represent each clades consisting of 3 (HSP70) and 4 (DnaK) identified NbHSP70s, respectively (Figure 4.). *In planta* physical interactions between CE86 with these two NbHSP70s were confirmed using co-immunoprecipitation (co-IP) assay with molecular tagged (GFP::HSP70, GFP::DnaK, and 3xFLAG::CE86) constructs in *N. benthamiana*. However, no direct interactions were identified between CE86 and both HSP70 homologs in the yeast-two hybrid system (Lee *et al.,* unpublished).

Effector target proteins usually function as positive regulators of plant defense, and NbHSP70c (homolog of our target protein HSP70 1A/1B) were previously reported as essential components for INF1-mediated cell death and also associated with plant defense (Kanzaki *et al.*, 2003). Therefore, to investigate

whether NbHSP70 and DnaK could function for plant defense against *P. infestans*, pk7-GFP::NbHSP70 or DnaK were transiently expressed on half of *N. benthamiana* leaf, and pk7-GFP::empty were infiltrated on the other half of leaf as a negative control via agro-infiltration 24 hours prior to *P. infestans* zoospore inoculation. Average lesion size were significantly decreased in target protein expressed regions, compared to empty vector (Figure 5). These results suggest that NbHSP70 and DnaK have own role in plant basal defense. And these results correlated with previous reports that HSP70 play role for basal defense in both arabidopsis and *N. benthamiana* (Jelenska *et al*., 2010; Kanzaki *et al*., 2003).

Table 3. Putative protein targets of CE86 in host plant *N. benthamiana* identified from *in planta* immunoprecipitation assay

Gene Id	Gene descriptions
552954066	MIP1.1b
*Niben101Scf00117g02019.1	Chaperone protein DnaK
Niben101Scf01999g06011.1	Chaperone protein DnaK
Niben101Scf02078g06002.1	60 kDa chaperonin
Niben101Scf02842g00014.1	60 kDa chaperonin 3
Niben101Scf03197g06003.1	60 kDa chaperonin 1
Niben101Scf03396g01003.1	60 kDa chaperonin
Niben101Scf03964g04005.1	Tetratricopeptide repeat protein 1
Niben101Scf04174g00007.1	Receptor-like protein kinase 2
Niben101Scf04364g01014.1	Heat shock 70 kDa protein 1
Niben101Scf04886g05003.1	Heat shock 70 kDa protein 1B
Niben101Scf09363g00018.1	60 kDa chaperonin 2
Niben101Scf09552g01001.1	Chaperone protein DnaK
*Niben101Scf12868g00008.1	Heat shock 70 kDa protein 1A/1B
Niben101Scf13703g01006.1	Heat shock 70 kDa protein 6
Niben101Scf29076g00011.1	60 kDa chaperonin 1

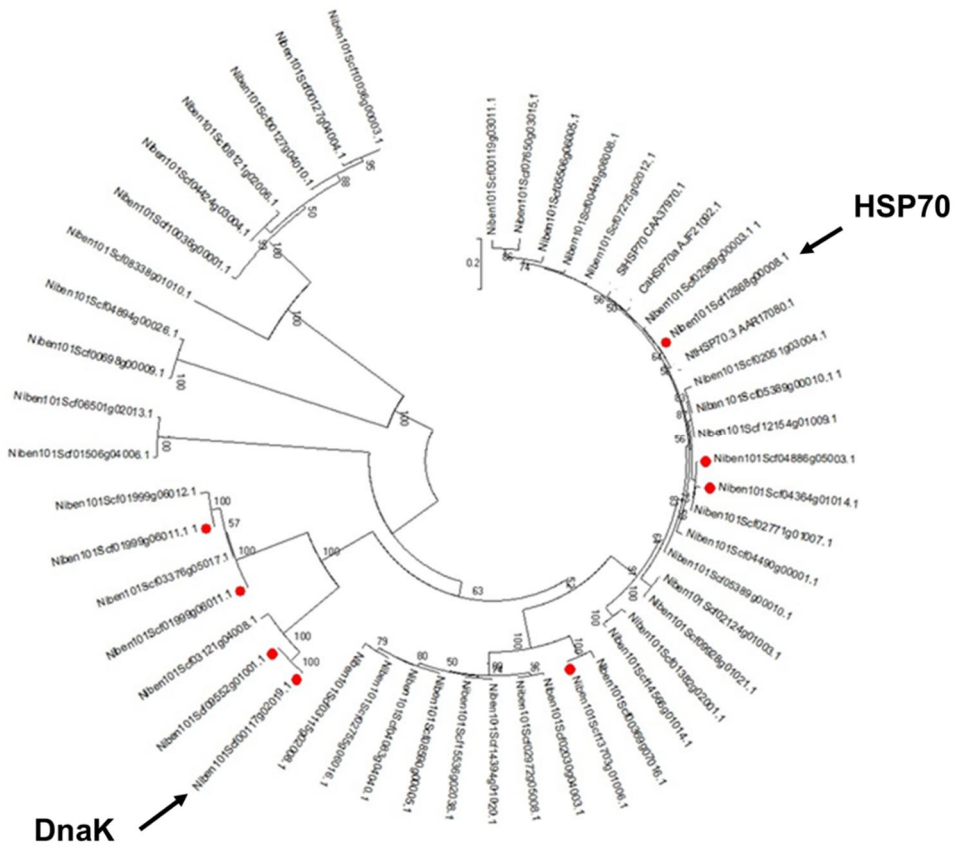


Figure 4. Phylogenetic tree of HSP70 family in *N. benthamiana*

Dots indicate NbHSP70 homologs which identified from LC-MS/MS result. HSP70 and DnaK which homologs selected for further studies are pointed with black arrows.

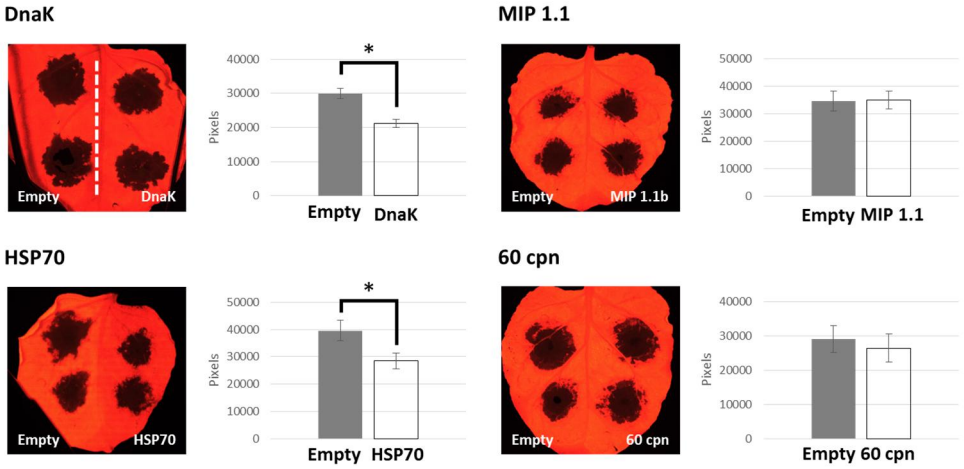
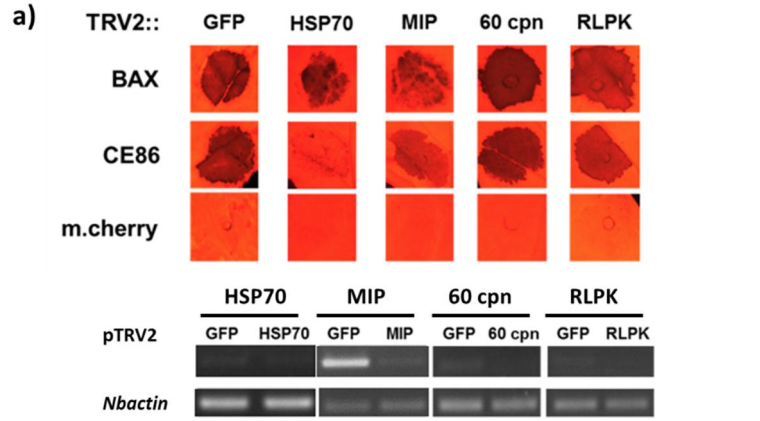


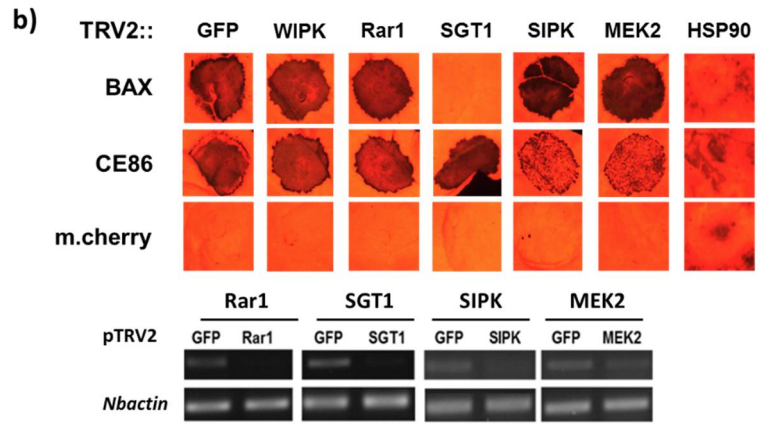
Figure 5. Expression of NbHSP70s decrease *P. infestans* lesion size on *N. benthamiana*

Agrobacterium carrying CEs were transiently expressed on half of *N. benthamiana* leaves with empty vector as a negative control on another half of leaves 24 hours prior to inoculation of *P. infestans* zoospores ($1.0x10^5$ zoospores/ml). Significantly decrease in lesion size was observed on HSP70 and DnaK infiltrated regions.

CE86-induced cell death is associated with HSP70 and MAPK cascades (SIPK and MEK2) in *N. benthamiana*

To investigate the effect of putative host targets on the CE86-induced cell death, several genes of putative protein targets identified from LC-MS/MS (Table 3.) were silenced by VIGS and CE86 were agro-infiltrated on silenced leaves. Most of silenced plants shown no different phenotypes from GFP silenced plant, but, HSP70 and DnaK silencing caused dwarfism and chlorophyll bleaching, respectively on *N. benthamiana* (Figure 6c). As a result, CE86-induced cell death was suppressed in HSP70 silenced *N. benthamiana* plants (Figure 6a). This results suggest that the putative host target protein, HSP70 is required for CE86-induced cell death. In addition, molecular components related with defense response were also silenced to investigate the signaling of CE86-induced cell death. As a result, CE86-induced cell death was compromised in SIPK and MEK2 silenced plants, which are components of MAPKs cascades (Figure 6b). Various studies have shown that MAPKs cascades are related to HR-like cell death and resistance response (Katou, Yamamoto, & Yoshioka, 2003; King *et al*., 2014; Sharma *et al*., 2003). According to these results, CE86-induced cell death is associated with HSP70 and MAPK signaling cascades of *N. benthamiana* which components are related with plant resistance.


a)
TRV2::
GFP
HSP70
MIP
60 cpn
RLPK
BAX
CE86
m.cherry
HSP70
MIP
60 cpn
RLPK
pTRV2
GFP HSP70
GFP MIP
GFP 60 cpn
GFP RLPK
Nbactin


b)
TRV2::
GFP
WIPK
Rar1
SGT1
SIPK
MEK2
HSP90
BAX
CE86
m.cherry
Rar1
SGT1
SIPK
MEK2
pTRV2
GFP Rar1
GFP SGT1
GFP SIPK
GFP MEK2
Nbactin

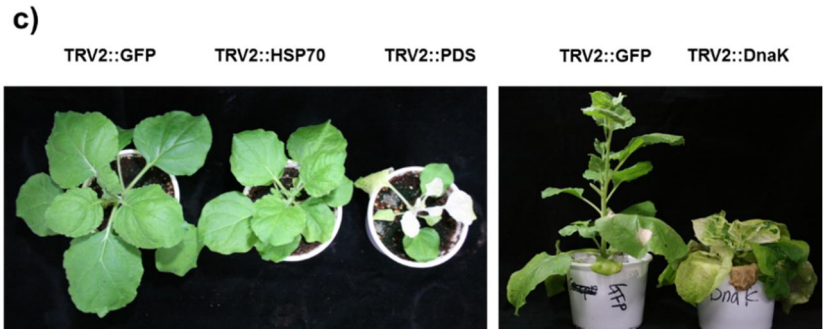

c)
TRV2::GFP
TRV2::HSP70
TRV2::PDS
TRV2::GFP
TRV2::DnaK

Figure 6. Silencing of NbHSP70s and MAPK signaling components reduced CE86-induced cell death on *N. benthamiana*

(a) Putative host target proteins and (b) general cell death/defense related components were silenced using TRV1 and TRV2 vector-mediated VIGS system in *N. benthamiana*. pkw::*CE86* was infiltrated on silenced leaves.Apoptotic cell death inducer Bax and m. cherry were used as positive and negative controls, respectively. Infiltrated leaves were detached at 4~5 dpi, depends on development of cell death phenotype on GFP-silenced plants, and photographs were taken under UV-light. (c) Phenotypes of HSP70 and DnaK silenced *N. benthamiana*.

Discussion

P. infestans contains hundreds of RXLR effector coding genes in genome, which regarded essential components for virulence, and several of them, could recognized by resistance genes and trigger immune responses in plants. In this study, to investigate non-host response of pepper against *P. infestans* in terms of ETI, 26 RXLR effectors were screened based on cell death phenotypes in non-host peppers and *N. benthamiana*. As results, 8 CEs exhibited HR-like cell death on pepper as potential Avr gene candidates. Among them, CE31, CE66 and CE86 were selected as candidates for identifying target proteins required for cell death phenotypes of these effectors on pepper or *N. benthamiana*.

Virulence test was performed to investigate whether candidate effectors, CE31, CE66 and CE86 could function during *P. infestans* infection. As a result, differential effects were observed in CE31, CE66, and CE86 expression on *P. infestans* growth in *N. benthamiana* (Figure 3). In case of CE86, lesion sizes were significantly enhanced compared to EGFP. However, possibility that cell death inducing activity of pCAMBIA2300::*CE86* could positively function for lesion size of *P. infestans* could not be completely excluded. To deal with this problem HIGS (host-induced gene silencing) method could be helpful. HIGS is RNAi technique for silencing pathogen genes which induced by accumulated ds/hairpin RNA-derived siRNA molecules transfer from host plant to pathogen (Nowara *et al*., 2010). Indeed, Vega-arreguín *et al*. reported that *Phytophthora capsici* growth was enhanced on *N. benthamiana* and *N. tabaccum* when *PcAvr3a* targeting hairpin construct expressed (Vega-arreguín *et al*., 2014). Similarly, HIGS could be

exploited for silencing CE86 to observe the contribution of CE86 to *P. infestans* virulence without CE86-mediated cell death activity. On the other hands, in cases of CE31 and CE66, average lesion sizes were decreased due to expression of effectors. Several researches reported about lesion size decreasing of hemi-biotrophic pathogens due to transient expression of specific effectors. Bacterial type-three effector HopPtoN, and oomycetes CRN effector PsCRN115 expression suppress lesion size of *P. syringae* pv. *tabaci* and *P. capsici*, respectively. Interestingly, these two effectors are also investigated to suppress defense-related HR (Zhang *et al.*, 2015; López-Solanilla *et al.*, 2004). Likewise these effectors, CE31 and CE66 could function as cell death suppressors which secreted during early phase of infection. However, in our experimental conditions (Figure 3) CE31 and CE66 were transiently expressed until 4~6 dpi. Therefore, these effectors could negatively function on later necrotic stage of *P. infestans*. On pepper, candidate CEs cannot be used for virulence test likewise in Figure 3, because candidate CEs trigger HR-like cell death on every tested pepper accessions when transient-expressed by agro-infiltration (Figure 1). Exploiting bacterial TTSS system and monitoring effects of CEs on bacterial growth could be alternative method to investigate role of candidate CEs in non-host pepper. As mentioned in literature review, similar approach was carried out with *P. syringae* expressing *Hyaloperonospora arabidopsis* RXLR effector on arabidopsis (Fabro *et al*., 2011). Likewise, on pepper, *Xanthomonas campestris* could be exploited for similar experiment.

To identify non-host resistance genes of pepper recognizing CE31, and 66, co-expression was performed with 415 cloned NLR encoding genes of CM334

pepper on *N. benthamiana* (Table 3). However, none of putative R genes induced HR cell death on co-infiltrated spot. From these results, several possibilities can be inferred. First, CEs could interact with other R genes, which are not involved in our 415 NB-LRR sets. While NB-LRR coding genes were predicted from high-quality CM334 pepper genome, but there are chronic limitations of computational prediction of NB-LRRs because these gene family usually clustered and exhibited high level of polymorphisms (Jupe *et al*., 2012). Second, several NB-LRR genes reported as requiring additional components for defense responses, such as RPS4 and RRS1, and RGA4 and RGA5 (Cesari *et al*., 2014; Narusaka *et al*., 2009). These NB-LRR genes forms complex with other NB-LRR genes (pairing) or indirectly recognize effectors by monitoring target proteins of their corresponding effectors (guard & decoy model) (Hoorn & Kamoun, 2008). Therefore, Table 3 could be explained as CE31 and 66 recognizing pepper R genes function in a complexed manner, not a one-to-one interaction. To deal with these difficulties, identification of target proteins (host target) of CE31 and 66 in pepper could be alternative method such as co-IP.

On the other hand, CE86 cannot be exploited for co-expression assay because CE86 induced cell death on *N. benthamiana* (Figure 2). Therefore, potential host target proteins were identified to investigate CE86-mediated cell death mechanisms by analyzing protein interactions in *N. benthamiana.* According to co-IP results, two NbHSP70 homologs (HSP70 and DnaK) were obtained as potential host targets of CE86. In previous researches, HSP70 reported as its own role for basal defense and also reported as host target of bacterial effector HopI1 and *Xanthomonas* type III effector AvrBsT (Jelenska *et al*., 2010; Open, 2015).

Likewise, we confirmed that our candidates negatively affect *P. infestans* growth on *N. benthamiana* (Figure 5), and involvement of HSP70 on CE86-mediated cell death (Figure 6). These results support that CE86-mediated cell death could be related with defense of *N. benthamiana*. Interestingly, CE86 induced cell death on both host *N. benthamiana* and non-host pepper (Figure 1). If so, CE86 could also interact with similar target proteins in non-host pepper likewise in *N. benthamiana* such as HSP70, and therefore, target protein identification of CE86 in non-host pepper could be helpful for understanding non-host resistance of pepper against *P. infestans*. However, the actual biological meaning of CE86-induced cell death and the relationship between HSP70 and CE86 needs to be further investigation. And observing differentiation of *P. infestans* growth on HSP70 silenced plant could be one method to investigate relationship between HSP70 and CE86.

In this paper, multiple candidate of effectors which possibly involved in NHR of pepper against *P. infestans* were screened. These selected effectors could be exploited to identify target proteins that recognize core effectors of non-adapted pathogens and could helpful for underpin the mechanism of NHR.

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국문 초록

비기주 저항성 (Non-host Resistance)는 한 종의 식물이 한 종의 병원균에 대하여 보이는 절대적이고 가장 보편적인 저항성 반응이다. 이런 비-기주 저항성 반응의 분자적인 기작은 아직 명확히 밝혀져 있지 않지만, 저항성 유전자 (R gene)가 병원균이 식물의 생리를 조절하여 번식하기 위해 식물 체내로 분비하는 병원성 단백질 (Effector)을 인지하여 일어나는 Effector-triggered immunity (ETI) 반응이 주요한 역할을 할 것이라고 논의되고 있다. 본 논문에서는 감자역병균의 주요 effector 57 개를 사용하여 비-기주 식물인 고추와 기주 식물인 담배에서 ETI 의 결과라고 생각되는 세포사멸 반응을 관찰하였고, 여기서 얻어진 비-기주 저항성에 관련되어 있을 것이라고 생각되는 effector 들을 이용하여 고추에서의 저항성 유전자 (Nucleotide-binding Leucine Rich Repeat, NLR) / 담배에서의 target 단백질을 동정해내는 실험을 수행하였다. 그 결과, 고추에서는 effector 와 1:1 로 반응하는 저항성 유전자를 동정해내지는 못하였지만, 같은 가지과 (Solanaceae) 식물인 담배에서는 CE86 의 target 단백질로서 HSP70 를 밝혀내었다. 즉, 같은 가지과 식물인 고추에서도 비슷한 상호작용이 있을 것이라 기대할 수 있고, 결론적으로 고추의 감자역병균에 대한 비-기주 저항성에 대한 실마리를 제공하였다고 볼 수 있다.