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Potide-G Derived from Potato (*Solanum tuberosum* L.) Is Active against Potato Virus Y^O (PVY^O) Infection

Giri Raj Tripathi,[†] Jaekyun Park,[†] Youngmin Park,[†] Indeok Hwang,[†] Yoonkyung Park,[†] Kyung-Soo Hahm,[‡] and Hyeonsook Cheong*,[†]

Department of Biotechnology and BK21 Research Team for Protein Activity Control, and Research Center for Proteineous Materials (RCPM), Chosun University, Gwangju 501-759, Republic of Korea

A PVY^O virus-resistant potato (*Solanum tuberosum* L. cv. Golden Valley) was identified, and further, from its tubers, a small (5.57 kDa) antiviral peptide potide-G was isolated. Application of potide-G on virus susceptible potato (cv. Winter valley) expressed robust resistance to PVY^O infection and showed no virus infected morphology. We found that PVY^O infection spreads up completely within 3 days post inoculation (dpi) in susceptible cultivar. PVY^O was more accumulated toward the basal leaves, when infection occurred longer. Combined results of morphology of PVY^O infection, ELISA, RT-PCR, and real-time PCR showed the resistance to the PVY^O infection depends on the expression of *Ry* gene. Indeed, the real-time PCR result showed that the *Ry* gene up-regulated to 3 times higher in PVY^O infected cv. Golden valley. Golden crude protein was found to be active against PVY^O infection in the in vivo test. In addition, application of potide-G in a virus susceptible potato potently reduced the viral infection actively with 50 times lower concentration than that of the Golden protein. Further identification of a host-specific resistant gene in a plant and the peptide derived from it offers new opportunities for the development of novel bio-pesticides against plant virus.

INTRODUCTION

PVY^O is a common strain that belongs to the necrotic group of Potato virus Y (PVY) (*1, 2*), which causes the primary symptoms of necrosis, mottling and yellowing of leaves that leads to premature death (*3*). The disease, which is tuber and aphid transmitted, is very harmful and leads to a huge loss in tuber yield (*4*).

Movement of the plant virus from the site of infection to the distal sites within a plant is generalized as short distance (cell-to-cell) and long distance (systemic or phloem-dependent) movement (*5, 6*). In short distance movement, virus is replicated in the infected cells, and then the infectious material is supplied cell-to-cell through plasmodesmata within the initially infected leaf (*7, 8*). Long distance movement is a phloem-dependent movement of a virus translocation from the infected site through the stem to the distal uninfected site that occurs through several vasculature-associated cell types, sieve elements, typically the phloem (*9, 10*).

The two main types of resistance to PVY in potato are extreme resistance (ER) and hypersensitive resistance (HR) (*11, 12*). ER strongly suppressed virus accumulation in infected cells, and no visible symptoms are detectable on inoculated plants

(*13*). HR may prevent the spread of virus within and from the inoculated leaf and develop necrotic lesions at the initial site of infection. HR could be activated but fails to restrict virus movement in plant tissues, which results in the development of larger necrotic lesions, lethal necrosis in the inoculated leaf (*14, 15*). Different methods including scoring the symptoms observed on inoculated leaves and on whole plants can be used to monitor the virus infection in a plant. Methods for locating the potato viruses in defined parts of the plants include the serological (ELISA) (*16*), RT-PCR (*17*), and real-time PCR (*18*).

The presence of a rigid cell wall in plants is itself passive defense against pathogens. When plants get mechanical or biological injuries, it resists assault by exhibiting an active defense mechanism upon the specific recognition of pathogen. Plants produce several types of proteins that mediate defense against pathogens and invading organisms, including ribosome-inactivating proteins (*19*), lectins (*20*), protease inhibitors (*21*), and antifungal proteins (*22*). Plant defense peptides have been long considered to play a key role against the plant pathogens. It acts both as a part of preexisting, developmentally regulated defenses barriers and as a component of the defense responses induced upon infection.

We report here on a novel plant antiviral peptide, designated potide-G, that has been isolated from potato (cv. Golden valley) tubers and is active against the PVY^O infection. Real-time PCR assay analyzed the relative changes that occurred in gene expression of the targeted *CP* gene after the appropriate

Table 1. Lists and Description of Primers

| target	PCR type	name	orientation	sequence 5′–3′	length (bp)
CP gene	RT-PCR	YCPF	forward primer	5′-AGGCACATCTGGGACACATACTGTGCCGA-3′	690bp
		YCPR	reverse primer	5′-TGACTCCAAGTAGAGTATGCATACTTGGA-3′	
	real-time	YCPRF	forward primer	5′-TTGGTGCATTGAAAATGGAA-3′	185bp
		YCPRR	reverse primer	5′-TTGCCTAAGGGTTGGTTTTG-3′	
Ry gene	RT-PCR	Ry-1F	forward primer	5′-GATGGCATCATCATCTTCTTCTTCTGA-3′	480bp
		Ry-1R	reverse primer	5′-CTTAGAGCTGTGACCTTTGGTTTCTTAGA-3′	
	real-time	Ry-1RF	forward primer	5′-AGGCACCTGATGGCTCGTAG-3′	154bp
		Ry-1RR	reverse primer	5′-TGAAATGCTTGTCTCTCGTTGTTG-3′	
actin		actinF	forward primer	5′-GGCGATGAAGCTCAATCCAAACG-3′	495bp
		actinR	reverse primer	5′-GGTCACGACCAGCAAGATCAAGACG-3′	
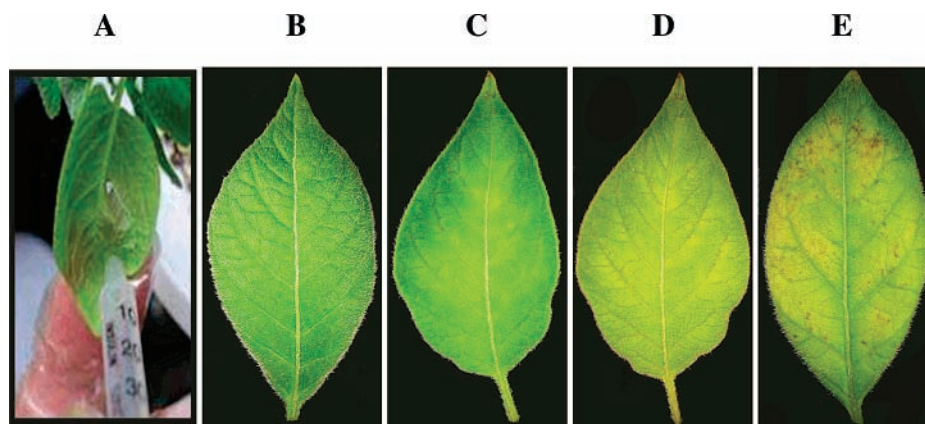
Figure 1. Phenotypic responses of PVY[O] infection (cv. Winter valley): (**A**) Inoculation of PVY[O] using a syringe. (**B**) Mock inoculated control. (**C**) First visible symptoms appeared in 7 dpi. (**D**) Yellow mosaic is increasing, and scattered necrosis appeared along the leaf vein (14 dpi). (**E**) Several local lesions and leaf spots developed on most of the leaf lamina (21 dpi).

exogenous treatment of potide-G. In parallel, the phenotype of viral infection differentiation in peptide-treated and non-treated plants was also observed. In addition, we quantified the up-regulation of *Ry* gene in extreme resistant potato after the PVY[O] infection.

MATERIALS AND METHODS

Plant Material, Growth Conditions, and Virus Inoculation. Healthy plants of different potato cultivars, Early valley, Gogu valley, Purple valley, Winter valley, Taedong valley, Summer valley, Taebok valley, Jopung valley, Juice valley, Rchip valley, Golden valley, and Superior valley, were obtained from Potato Valley Co. Ltd., Korea (http://www.potatovalley.com), and propagated in tissue culture, and the plantlets with root were transferred to autoclaved soil (Mix 5 Soil, Sun Grow Horticulture, Canada). Each plant was watered twice a week with 50 mL of distilled water and once with 50 mL of mineral nutrient solution (0.1% Hyponex) (*3*). Potato plants were grown in the growth room at $21 \pm 1°C$ under a 16/8 h (light/dark) photoperiod and relative humidity of $75 \pm 1\%$. Healthy leaves of the 3−4 weeks soil grown plants were selected for PVY[O] inoculation. Virus inoculums prepared from 1 g of secondarily PVY[O] infected leaves of tobacco cv. Burely21 plants were grown in tissue culture, ground in 3 mL of 20 mM sodium phosphate buffer (pH 7.6), and inoculated with the help of syringe. After 15 min of inoculation, leaves were rinsed with distilled water. The non-inoculated plant of each cultivar was used as a control. Following the inoculation, plants were examined daily for symptoms observation. The leaves from the inoculated and non-inoculated plant of each cultivar were harvested 3 and 14 days after inoculation (dpi). The leaves of inoculated, upper first and third, and lower first and third leaves from the inoculation were harvested separately and tested for PVY[O] activities on ELISA, RT-PCR, and real-time PCR.

Protein Preparation. The crude protein was isolated, and the frozen leaf material from the *Ry* gene-rich potato was ground to a homogeneous powder in liquid nitrogen with a mortar and pestle. The homogenate was thawed and incubated with extraction buffer containing 50 mM

Tris-HCl (pH 7.4), 1 mM EDTA, 10 mM NaCl, 5 mM $MgCl_2$, 1% Triton X-100, and 2% 2-mercaptoethanol at 4 °C for an hour. The mixture was clarified by centrifugation at 15 000*g* for 15 min at 4 °C. The supernatant was pooled and freeze-dried to obtain the concentrate dry powder. Obtained protein powder was dissolved in autoclaved distilled water before use.

Purification and Characterization of Potide-G. The crude cell wall extract was obtained from the potato cv. Golden valley tuber material using methods as previously described by Kim et al. (*23*) and loaded on a DEAE-cellulose reversed-phase column (Vydac, 4.6 × 250 mm) on an HPLC system (Shimadzu, Japan). Peptides that passed through the column unadsorbed were dissolved in 0.1% (v/v) trifluoroacetic acid (TFA) in HPLC grade water (Solvent A) and loaded onto a C_{18} RP-HPLC column in equilibrium with 0.1% TFA. The peptides were eluted with a linear gradient (2% increase/min) of acetonitrile (30% to 60%) containing 0.1% trifluoroacetic acid at a flow rate of 1 mL/min. The effluent was monitored at 230 nm, and the fractions for each peak were pooled and dried in a freeze-dryer. The major peak was resubjected to another round of purification. The resulting peak fraction was collected and assayed for antiviral activity.

Protein and Peptides Treatments. Potato highly susceptible to PVY[O] infection (cv. Winter valley) grown as described above was used for this test. 500 μg of protein extracted from leaves of cvs. Golden valley and Gogu valley, and 10 μg of peptides Analogue-3 (*24*) and potide-G were applied separately by cotton swab on the dorsal side of the healthy leaf of a 3-week-old plant. After 10 min of protein/peptide treatment, the plants were infected with PVY[O] virus as described above. PVY[O] infected cv. Winter valley was used as a control.

Double Antibody Sandwich ELISA (DAS-ELISA). DAS-ELISA was used according to the protocol provided by Agdia, Inc., Elkhart, IN (http://www.agdia.com). All reagents were obtained commercially (Agdia, Inc., USA) and were applied at 100 μL per well in microtiter plates (Nunclon delta surface, Denmark). Leaf samples were extracted in a 1:10 (v/v) dilution of general extraction buffer (Agdia, Inc., ACC 00955), and lysate was centrifuged at 15 000*g* for 15 min at 4 °C. Obtained supernatant was added to microtiter plate as an antigen (*3*).

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Table 2. Analysis of PVY^O Infection by Combining the Results of Morphology, DAS-ELISA, and RT-PCR[a]

potato cultivars	PVY^O infection[b] (phenotype)	ELISA[c]	RT-PCR CP gene	RT-PCR Ry gene
Early valley	2	3	2	
Gogu valley	2	2		1
Purple valley	2	2		1
Winter valley	3	3	3	
Taedong valley	1	1		2
Summer valley	1	1		2
Taebok valley	3	3	2	
Jopung valley	1	2		2
Juice valley	2	2		1
Rchip valley	1	1		2
Golden valley				3
Superior valley	1	2		1

[a] Legends: 3 = high, 2 = moderate, 1 = less. [b] Phenotypes were observed after the 21 dpi of PVY^O inoculation. [c] Tripathi et al. (*3*).

All of the samples were measured spectrophotometrically at an absorbance of 650 nm using an ELISA plate reader (Molecular Devices, USA). All assays were performed in triplicates. Mock inoculated cv. Winter valley was used as a control.

RNA Isolation and cDNA Synthesis. Total RNA was extracted with Trizol (Life Technologies) from 100 mg of potato leaf sample following the manufacturer's protocol. The concentration of extracted RNA was quantified at the absorbance of 260 nm in a spectrophotometer (Smart Spec 3000, BIO-RAD, USA). All of the extracted RNA was diluted to 1 μg/μL. cDNA was synthesized using Superscript II reverse transcriptase (Life Technologies). 2 μg of total RNA and 1 μL of the oligo dT (500 μg/mL) were mixed in a reaction tube, heated at 70 °C for 10 min to inactivate the sample, and then chilled on ice quickly. 5X first strand buffer and 0.1 M DTT were added, and the mixed contents of the tube were gently incubated at 42 °C for 2 min. 1 μL (200 units) of Superscript II was added to the tube, incubated at 42 °C for 50 min, and the reaction stopped by inactivation heating at 70 °C for 15 min (*3*). Synthesized cDNA was stored at −20 °C for further use.

Oligonucleotide Primers. The design of primers was performed to avoid primer−dimers or self-priming formation using Prime3 software. All oligonucleotide primers were synthesized by Takara (Japan). The real-time PCR and RT-PCR primer sequences used in this study are shown in **Table 1**. The primer pair of YCPF/YCPR and YCPRF/YCPRR was designed to amplify the coat protein gene of PVY in potato leaf based on deposited sequences (Gene Bank accession no. NC_001616). The Ry-1F/Ry-1R and Ry-1RF/Ry-1RR primers were used to amplify the expression of *Ry* gene in potato leaf (Gene Bank accession no. AJ300266).

RT-PCR Assay. All of the RT-PCR amplifications were performed in 20 μL reaction volume, with 200 ng of cDNA, added to 1x Taq buffer, 0.25 mM of dNTPs, 0.5 μM of each forward and reverse primers, and 1 unit of Taq DNA polymerase (*3*). *CP* gene was amplified with a denaturation of 5 min at 94 °C; 30 cycles at 94 °C for 30 s; annealing of 30 s at 57 °C and extension of 1 min 30 s at 72 °C, with a final extension of 5 min at 72 °C. Similarly, the *Ry* gene was amplified with a denaturation of 3 min at 94 °C; 30 cycles at 94 °C for 30 s; 55 °C for 30 s annealing and 72 °C for 1 min extension, with a final

extension of 5 min at 72 °C. PCR products were electrophoresed in a 1% agarose gel containing 0.5 mg L^{-1} ethidium bromide (EtBr) and observed under ultraviolet light.

Quantitative (Real-Time) PCR Assay. Each 20 μL reaction mixture contained the following ingredients: QuantiTect SYBR Green PCR Master Mix (HotStarTaq DNA Polymerase, QuantiTect SYBR Green PCR Buffer, dNTPs including dUTP for optional uracil-*N*-glycosylase treatment, SYBR Green I, and ROX, a passive reference dye), 5 pmol of each primer, 200 ng of template cDNA, and RNase-free water. All of the quantitative PCRs were performed as follows: 15 min polymerase activation at 95 °C and 40 cycles of denaturation at 95 °C for 10 s, annealing at 54 °C for 20 s, extension at 72 °C for 30 s and a final extension at 72 °C for 5 min in a fluorometric thermal cycler (Rotor-Gene 3000, Corbett, Australia). Real-time PCR assay was performed in triplicate. The expressed relative concentrations were analyzed using Rotor-Gene Real-Time software 6.0.

RESULTS AND DISCUSSION

A potato cv. Golden valley, extremely resistant against PVY^O infection, was identified among the Korean potato valley cultivars. In the first screening step, potato plant was infected with PVY^O virus at six to eight leaf stages (about 3 weeks plant on soil). The entire inoculation was performed with the help of syringe without damaging the leaf surface (**Figure 1A**). Cv. Winter valley was found highly susceptible to the PVY^O infection with the development of local disease symptoms, expressed as appearance of leaf bunching and crinkling, mild to strong yellow leaf mosaic, necrotic veins, leaf spots, and leaf dropping. The first visible symptoms were observed as green spots and later rugose mosaic to leaf spot and yellow mosaic (**Figure 1C−E**). The yellow mosaic symptoms increased until the third week of infection during the study period of 45 days. Yet the infected cv. Golden valley did not show any symptoms of infection over the entire life of the plant (data not shown). However, the level of PVY^O infection was varied from cultivar to cultivar (**Table 2**).

We also performed the RT-PCR amplification to identify the *Ry* gene-rich potato cultivars. All of the RNAs were prepared from PVY^O infected potato leaves and used primers Ry-1F and Ry-1R designed from the *Ry* gene coding region (**Table 1**). A 480bp band corresponding to the expected size was over-expressed differently in each cultivar. It was highly expressed in cv. Golden valley and then cv. Summer valley; moderately in cvs. Taedong valley, Jopung valley; and less in Gogu valley, Purple valley, Juice valley, and Superior valley (**Figure 2**). We also amplified PVY^O-specific *CP* gene using primers YCPF and YCPR (**Table 1**). A 690bp band corresponding to the expected size of the sequence including both primers was observed in the cultivars Winter valley, Early valley, and Taebok valley (**Figure 2**). Finally, matrix analysis of the results of morphology, ELISA, and RT-PCR identified that the cv. Golden valley is extremely resistant to PVY^O infection, which is rich in *Ry* gene expression.

Next, we examined the directional movement of PVY^O whether it accumulates more in the upper or lower parts of the
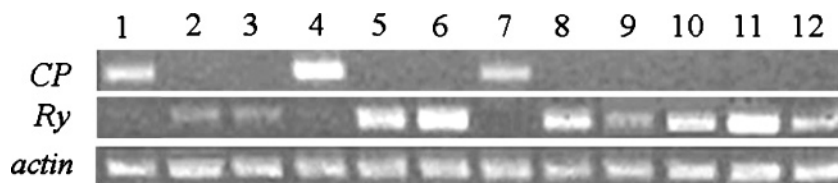

Figure 2. Comparative band intensities of virus resistant (*Ry*) gene and coat protein (*CP*) gene were detected on RT-PCR amplification using specific primers (**Table 1**). cDNAs were synthesized separately from RNA isolated from different cultivars of the PVY^O infected potato. Lane 1, cv. Early valley; lane 2, cv. Gogu valley; lane 3, cv. Purple valley; lane 4, cv. Winter valley; lane 5, cv. Taedong valley; lane 6, cv. Summer valley; lane 7, cv. Taebok valley; lane 8, cv. Jopung valley; lane 9, cv. Juice valley; lane 10, cv. Rchip valley; lane 11, cv. Golden valley; lane 12, cv. Superior valley.
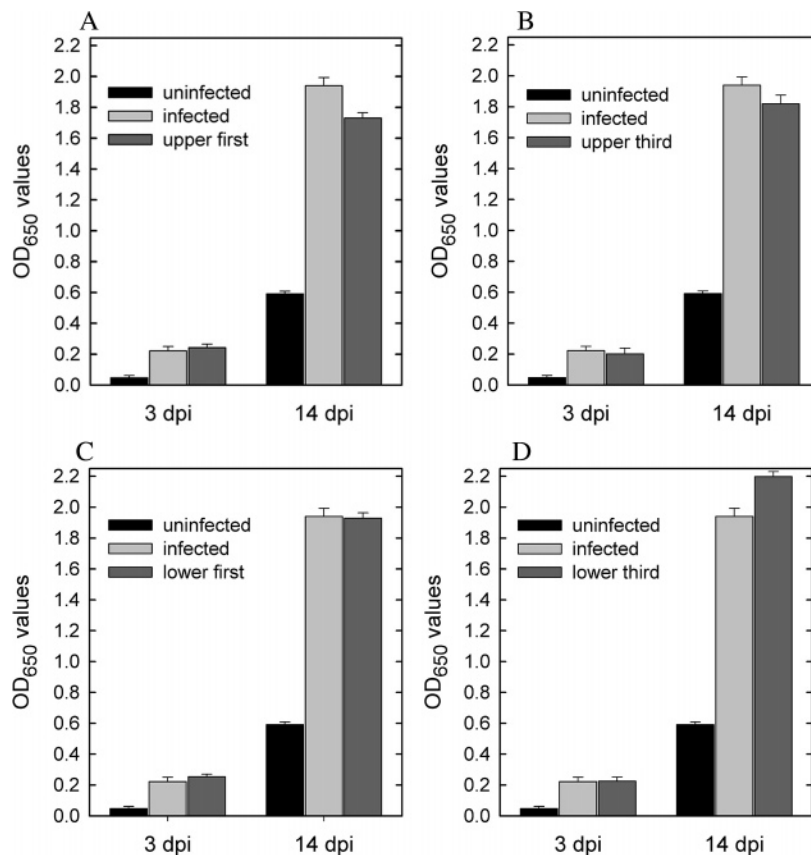
Figure 3. Detection of virus accumulation in potato leaves inoculated with PVY[O] using DAS-ELISA. ELISA (OD$_{650}$ values) was performed in 3 dpi and 14 dpi on the leaves of upper and lower direction from the infected leaf. PVY[O] infected cv. Winter valley was used as control. Plots average ELISA values are from the extracts of three different inoculated leaves and three independent replicated experiments. Standard errors are represented by error bars. (**A**) Upper first leaf, (**B**) upper third leaf, (**C**) lower first leaf, (**D**) lower third leaf.
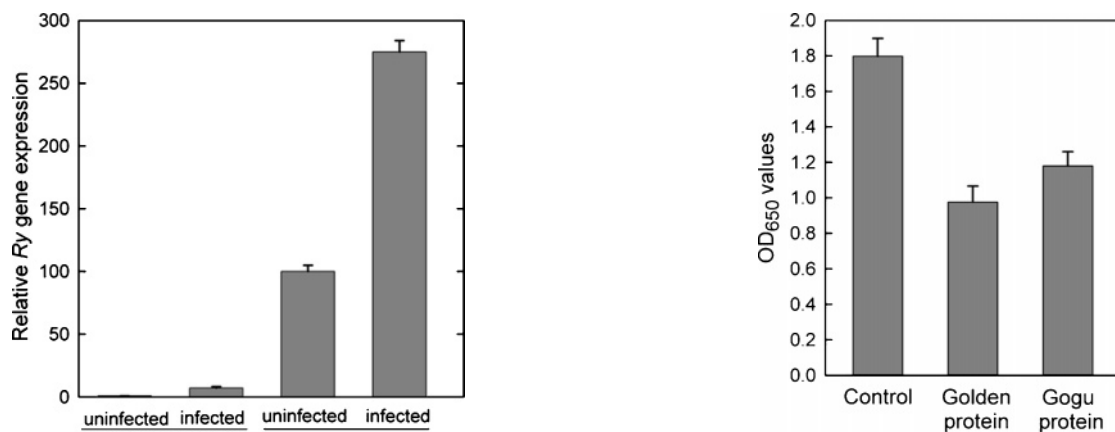

Figure 4. Relative expression levels of PVY[O] resistant *Ry* gene measured in virus inoculated and noninoculated leaves of virus susceptible cv. Winter valley and virus resistant cv. Golden valley. A 154bp fragment of *Ry* gene was amplified in real-time PCR using specific primer (**Table 1**). Amounts of cDNAs were calibrated using actin as reference. Data presented are the average and standard deviations of three independent replicated experiments.

Figure 5. Antiviral activity of the potato proteins against PVY[O] infection. Crude protein was extracted from leaves of potato cv.'s Golden valley and Gogu valley. Different concentrations of each protein were applied separately on the leaf of cv. Winter valley after the 10 min of PVY[O] infection (data not shown). After the 14 dpi, virus infection was detected using DAS-ELISA (OD$_{650}$). 500 μg of crude Golden protein was determined to be the amount effective against the PVY[O] infection. Data presented are the average and standard deviations of three independent replicated experiments.

plant. After the 3 dpi and 14 dpi of PVY[O] inoculation in susceptible cv. Winter valley, the first and third leaves of each plant in the upper and lower direction from the inoculated leaves were harvested food and tested for virus movement using DAS-ELISA. Sensitivities of ELISA showed that the PVY[O] infection was spread up parallel in average on all of the tested leaves within 3 dpi (**Figure 3**). Yet after the 14 dpi, the OD value of

ELISA showed that PVY[O] was accumulated much toward the basal part of the plant leaf (**Figure 3D**). Consistent with this finding, the PVY[O] is more accumulated in the lower parts when infection occurs longer.

To understand the up-regulation of (*Ry*) gene, we examined the mRNA expression level of *Ry* gene using real-time PCR
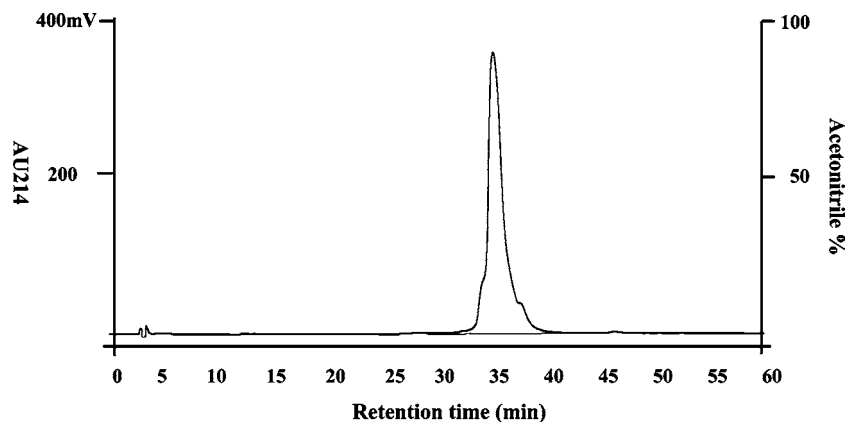
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Figure 6. Purification of potide-G. Reversed-phase HPLC fractionation of the potato tuber extract. The peptides were eluted with a linear gradient (2% increase/min) of acetonitrile (10% to 95%) containing 0.1% trifluoroacetic acid at a flow rate of 1 mL/min. The purified peptide was then again subjected to reversed-phase HPLC using a C$_{18}$ column. The effluent was monitored at 214 nm.

on RNA extracted from the leaves of PVY^O infected cv.'s Golden valley and Winter valley. The relative expression level of the *Ry* gene in PVY^O infected cv. Golden valley was found to be approximately 3 times higher on average than that of the uninfected control plant (**Figure 4**).

Later, we isolated the crude protein from highly and moderately resistant cv.'s Golden valley and Gogu valley, respectively, and treated the Winter valley and infected with PVY^O. After the 14 dpi, the treated leaves were harvested, and we examined the PVY^O sensitivities using ELISA. The application of each 500 μg of Golden protein reduced the PVY^O accumulation on average 50%, and Gogu protein was reduced by 38% (**Figure 5**). From these findings, we assumed that purified protein from *Ry* gene-rich potato cv. Golden valley may control PVY^O virus infection.

After that, we purified an antiviral peptide of the potato (cv. Golden valley) tuber extract using ultrafiltration and DEAE-cellulose and C$_{18}$ reversed-phase HPLC. In the first isolation step, ultrafiltration through a 10-kDa molecular weight cutoff membrane yielded two components: one with molecular masses >10 kDa and the other with molecular masses <10 kDa. In the second isolation step, <10-kDa samples were fractionated on DEAE-cellulose into an unadsorbed fraction 1 with antiviral activity and an adsorbed fraction 2 without activity (data not shown). Fraction 1 was subjected to further fractionation by C$_{18}$ reversed-phase HPLC. The major single peak, which we refer to as potide-G, was then further purified through two additional steps of C$_{18}$ reversed-phase HPLC. The relative molecular weight of potide-G was of 5.57 kDa, directly determined by MALDI-MS (**Figure 6**).

Next, we examined the antiviral activity of potide-G against PVY^O infection. The leaf of the cv. Winter valley was inoculated with PVY^O following the 10 min of treatment of potide-G. In this time, potato leaves were inoculated with high concentrations of PVY^O (10 mg/mL) to maximize the viral infection. After 14 days of potide-G treatment, the leaf samples that had been inoculated with PVY^O were subjected to real-time PCR for detection of the presence of virus. We also applied an antibiotic peptide, Analogue-3 (A3) (*24*), to compare the effectiveness of peptides. As a result, real-time PCR showed that the application of 10 μg of purified potide-G was sufficient to reduce 50% in average virus accumulation from the PVY^O infection in susceptible cultivar cv. Winter valley (**Figure 7**).

Plant virus spread fast up and down in the stem at approximately the same time (*2*). It is generally accepted that, in the first stage of infection, accumulation of virus and viral gene
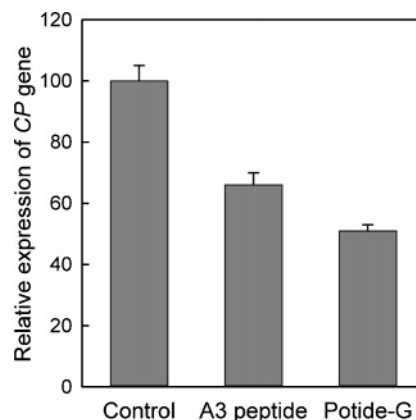

Figure 7. Real-time PCR (*CP* gene). Relative expression of *CP* gene measured in leaves after 14 days treatment of an antiviral peptide potide-G on PVY^O infected cv. Winter valley. Amounts of cDNAs were calibrated using the actin as reference. PVY^O infected cv. Winter valley was used as control, and an antimicrobial peptide Analogue-3 (A3) was used to compare the effectiveness against virus infection. Data presented are means and standard deviations of three independent replicated experiments.

products within the initially infected cells will subsequently spread to the neighboring cells (*6*). Once virus enters phloem tissues within the inoculated leaf, it moves rapidly (cm/h) toward the growing region or other food-utilizing parts of the plant (*25*). We found that PVY^O was accumulated more toward the basal parts of the leaves when infection occurred longer (**Figure 3**). This was probably because during and after the spreading of PVY^O it replicates in all cells and appeared in a source-to-sink pattern and flow of photoassimilates system (*6*). Solanaceae have both abaxial and adaxial phloem strands to correspond to the external and internal phloem of the stem, respectively (*26*). The abaxial phloem is associated with sugar export from the leaf to the other growing parts of the plant like tubers and roots (*27*). However, to understand more about directional movement of plant virus, a more detailed study using different molecular techniques such as imunocapture PCR, fluorescence ELISA, and frequent measurement would be appropriate.

Ry gene is a host-mediated virus-resistant gene in potato. It is accepted that host gene-mediated extreme resistance (ER) prevents virus multiplication at an early stage (*28, 29*) and hypersensitive resistance (HR) has a rapid defense that results in the death of a few cells (necrosis) at the site of infection, which prevents the infection from spreading further (*4, 30*). We

found that the different cultivars have different types of resistance (**Table 2**). The plants with resistance in virus accumulation are infectible, but the virus reaches relatively low concentration in the plant with moderate resistance (*31*). Therefore, we detected the mRNA expression level of *Ry* gene and identified the types of resistance. Sometimes, it is also accepted that the result of RT-PCR for virus detection depends on the quality of cDNA, which is determined by many factors (*17*). However, from this study, it is suggested that ELISA is a more sensitive tool for virus detection, but RT-PCR could be used as a complementary method, and conforming the cultivars is either virus susceptible or resistant.

Quantization of host-specific resistant gene is very important to know the host pathogen interactions. Recently, real-time fluorescent PCR is a most frequently used technique for the detection and quantification of viral particles in different parts of the plant (*18*, *32*). We identified *Ry* gene-rich cultivar and further isolated the potide-G from its tubers. The peptides isolated from potato tubers are generally found to be active against fungal and bacterial pathogens (*23*, *33*). Plant-resistant (R) gene encoded protein contains an N-terminal coiled-coil (CC) domain, a central nucleotide-binding site (NBS) domain, and a C-terminal leucine-rich repeat (LRR) domain. CC-NBS-LRR recognizes specific pathogen-derived products and initiates resistant response (*34*). We found that potide-G isolated form the cv. Golden valley exerts an antiviral effect against PVY^O infection (**Figure 7**). There is indirect evidence that the LRR may contribute to signaling as well as recognition specificity (*35*, *36*). However, it is generally accepted that the resistance (R) proteins mediate elicitor recognition and activate downstream signaling responses, leading to the disease resistance (*37*, *38*). Thus, further investigation is needed to understand the signaling response against the virus pathogen in plant.

In summary, we identified the *Ry* gene-rich potato (*S. tuberosum* L. cv. Golden valley) and isolated the peptide, potide-G, from its tubers. We also determined the virus movement and interaction of *Ry* gene against PVY^O infection. Potide-G showed potent antiviral activity against the PVY^O infection. Therefore, potide-G may serve as a potential candidate for designing future agrochemicals because of a potent effect toward PVY^O infection and the fact that it is environmentally friendly and cost-effective.

ACKNOWLEDGMENT

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