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# Expression of *G-Ry* Derived from the Potato (*Solanum tuberosum* L.) Increases PVY$^O$ Resistance

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In Solanaceae, potato virus Y$^O$ (PVY$^O$) is a widespread virus leading to severe damages such as necrosis, molting, and yield reduction. The resistance *Y* gene (*Ry* gene) of potato specifically confers resistance to PVY infection. Previously, potatoes resistant to PVY$^O$ infection were screened among the 32 Korean cultivars. 'Golden Valley' displayed the most resistance to PVY$^O$ infection. 'Golden Valley''s *Ry* gene (*G-Ry*) was cloned from 'Golden Valley', and the function was investigated. G-Ry protein contains 1134 amino acid residues and is structurally similar to the Y-1, which confers resistance to PVY infection in *Solanum tuberosum* subsp. *andigena*. To generate a PVY$^O$-resistant potato, the *G-Ry* gene has been introduced into 'Winter Valley', the cultivar most susceptible to PVY$^O$ infection among the 32 Korean cultivars. Transgenic 'Winter Valley' ('Winter Valley'-G) showed an increased resistance to PVY infection. This approach may ultimately lead to the development of a virus-resistant plant.

**KEYWORDS: *G-Ry*; PVY$^O$ resistance potato; *CP* gene; *Solanum tuberosum***

## INTRODUCTION

Potato virus Y (PVY), the type member of the genus *Potyvirus* of the Potyviridae family, is a widespread virus. The main strain of PVY is commonly subdivided into PVY$^O$, PVY$^C$, and PVY$^N$ (*1*, *2*). All main strains of PVY were discriminated by RT-PCR (*3*,*4*). Symptoms of PVY infection in Solanaceae (*Solanum tuberosum* L.) vary from mild to severe mottles on most hosts to a streak or leaf-drop streak resulting from long necrotic lesions along the veins on the underside of leaflets of some potato varieties (*5*, *6*). PVY$^N$ induces venal necrosis and mottling in *Nicotiana tabacum* and very mild mottling in the majority of potatoes (*7*). PVY$^C$ induces stripple streak symptoms on some potato cultivars (*8*). PVY$^O$ is the common or ordinary strain and induces primary symptoms such as necrosis, mottling, and yellowing of leaves and possibly premature death (*2*, *9*).

Potatoes infected with PVY exhibit hypersensitive resistance (HR) or extreme resistance (ER). Genes for HR and ER have been identified and are reported to originate from the related host species. Plants with HR evidence either partial necrotic lesions, which can prevent the infection from spreading further, or systemic necrosis. Conversely, host gene-mediated ER prevents viral multiplication at the early stages of infection, and the plant evidences either no symptoms or limited necrosis (e.g., pinpoint lesions, flecks, or localized stem necrosis) (*1*, *10*, *11*). Few sources of ER provided by dominant genes exist for certain potato viruses. Examples of durable resistance genes thus far include a dominant *Ry* gene, which confers ER to all strains of PVY in the potato (*12*−*15*). The most extensively utilized strategies for the control of viral disease have focused on the development of virus-resistant potato cultivars. Transgenic potato plants that expressed Y-1 under the control of the Cauliflower mosaic virus 35S promoter were developed, but these plants did not show significant resistance, and the plants were systemically infected and evidenced necrotic lesions with PVY (*16*).

Previously, the detection of a Y-1-like PVY$^O$ resistance gene in the Korean potato was expected. The inoculation of 32 Korean potato cultivars with PVY$^O$ was screened, and various resistance levels were detected (*17*). We determined that the 'Golden Valley' variant was the most resistant to PVY$^O$ infection among the 32 Korean potato cultivars; conversely, the 'Winter Valley' variant was identified as the most susceptible. We also observed sufficient results for antiviral activity from the application of the 'Golden Valley' protein and potide-G, a small (5.57 kDa) antiviral peptide isolated from the 'Golden Valley' cultivar (*18*). Here, we report the genetic transformation of the potato (cv. 'Winter Valley') with the *G-Ry* gene isolated from 'Golden Valley' potatoes, and the induction of increased resistance against PVY$^O$ infection. The findings of this study may help in the future development of virus-resistant plants.

## MATERIALS AND METHODS

**Plant Material and Growth Conditions.** The 'Winter Valley' and 'Golden Valley' potato variants were obtained from Potato Valley (Kwangwon province, Korea). The tubers were seeded in autoclaved soil. When the shoots grew to plants of 10 cm in height, the stem segments of the plant were transferred to Murashige and Skoog (MS) medium including 3% sucrose after sterilization with 30% Clorox. Plants propagated in vitro were utilized for transformation. All plants were grown in a growth room at 23 °C under long-day conditions.

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**Table 1.** Lists and Description of Primers

| target | primer name | nucleotide sequence (5′−3′) |
|---|---|---|
| *Ry* gene | RyF10 | 5′-ATCCCGGGATGGCATCATCATCTTCTTC-3′ |
| | RyIR | 5′-AAGCATGCAATATCTAGAAATATCTCTTGA-3′ |
| | RyIF | 5′-TTGCATGCTTCTTAAGAGGGAGAAAACAAA-3′ |
| | RyR10 | 5′-TACCCGGGCTCACATTGTCAACATCTTCAGT-3′ |
| | GdF7 | 5′-GAATCTTGCCTTATGCAGCCATTCA-3′ |
| | GdR3 | 5′-GATAACTCCATGTGCCTATAATCAT-3′ |
| *CP* gene | CPF2 | 5′-GACACATACTGTGCCGAGAATCAAGGCTA-3′ |
| | CPR2 | 5′-CATTCATCACAGTTGGCATCTCAGT-3′ |
| EF1α | EF1F | 5′-TCATCATCATGAACCATCCTGGCCAG-3′ |
| | EF1R | 5′-TCATCTTAACCATACCAGCATCACCGT-3′ |

**Table 2.** Potato Regeneration Medium

| medium | composition |
|---|---|
| stage I | MS salts, 0.5 g/L MES, 20 g/L sucrose, 0.4 mg/L IAA, 2.24 mg/L BAP, pH 5.8 |
| stage II | stage I medium, 50 mg/L kanamycin, 250 mg/L cefotaxime, pH 5.8 |
| stage III | MS salts, 0.5 g/L MES, 20 g/L sucrose, 2.24 mg/L BAP, 250 mg/L cefotaxime, pH 5.8 |
| infiltration | MS salts, 0.5 g/L MES, 20 g/L sucrose, pH 5.8 |

**RNA Preparation and cDNA Synthesis.** Total RNA was extracted from 100 mg of plant leaves with TRIzol reagent (Invitrogen) in accordance with the manufacturer's protocols. cDNA was synthesized with Superscript II reverse-trasncriptase (Invitrogen). Two micrograms of total RNA and 1 μL of oligo dT (500 μg/μL) were mixed in a reaction tube and heated for 10 min at 65 °C and then quick-chilled on ice. The enzyme was added to the tubes, and the tubes were incubated for 50 min at 42 °C. The reaction was halted by heating at 70 °C for 5 min. The prepared cDNA was utilized for *G-Ry* cloning and RT-PCR (reverse transcriptase Polymerase Chain Reaction) analysis.

**Cloning of *G-Ry*.** Two fragments of the *G-Ry* gene were amplified using two pair primers, RyF10/RyIR and RyIF/RyR10 (sequence shown in **Table 1**), from the cDNA of the 'Golden Valley' potato cultivar. The *Xma*I and *Sph*I restriction enzymes were utilized for the cloning of *G-Ry*. The two amplified fragments were cloned directly into the pUC19 vector. The construct was then digested with *Xma*I and *Sph*I restriction enzymes, and the fragment including *G-Ry* was subcloned into the binary vector, pBI121. All constructs were confirmed via PCR, digestion with restriction enzymes, and sequencing.

**Potato Transformation and Regeneration.** *Agrobacterium*-mediated transformation was conducted for transformation into the potato (*19*). *Agrobacterium* was grown for 16 h, pelleted, and resuspended in infiltration medium (contents shown in **Table 2**). Stem explants (approximately 5 mm long) without axillary buds were prepared from plants grown in vitro. The stems were then precultured on stage I medium (contents shown in **Table 2**) for 2 days. The precultured explants were then soaked for 10 min in infiltration medium. The explants cocultured with *Agrobacterium* were transferred to fresh stage I medium and cultured for 3 days in darkness. After 3 days of cocultivation, they were transferred onto stage II medium (contents shown in **Table 2**) for the screening of transgenic plants and cultured under long-day conditions for 10 days. They were then transferred onto stage III medium (contents shown in **Table 2**) for shoot regeneration. After primordial formation, the explants containing the primordial part were subcultured in hormone-free MS liquid medium with shaking.

**Virus Inoculation.** The ampule of the $PVY^O$ virus was obtained from the Plant Virus Genbank (Seoul Women's University, Korea). The $PVY^O$ sample was frozen immediately with liquid nitrogen and then finely ground to powder with a mortar. The powder including $PVY^O$ was thawed in sodium phosphate buffer (pH 7.0) for the inoculation of the virus. The leaves injected with $PVY^O$ using a syringe were inoculated mechanically. The apical leaflets of each plant were assessed for the detection of $PVY^O$.

**Double Antibody Sandwich ELISA (DAS-ELISA).** The direct double-antibody sandwich ELISA method (DAS-ELISA) (Agdia, Inc., Elkhart, IN) was employed in accordance with the instructions provided by Agdia Inc. Total proteins were extracted from the leaves of each plant with a general extraction buffer (material provided by Agdia Inc.). To test $PVY^O$ using DAS-ELISA, 100 μL samples, including 10 μg of protein, were plated onto anti-PVY coated 96-well plates (Agdia, Inc., 00210). The plates were incubated for 2 h at room temperature and washed seven times in PBST buffer. The enzyme conjugates were then dispensed into each test well, and the plates were incubated for 2 h at room temperature. The plates were washed eight times in PBST buffer. PNP substrate was added to each test well, and the plates were incubated for 1 h at room temperature. The samples were then observed spectrophotometrically at 405 nm with an ELISA Reader (Bio-Rad, Hercules, CA). All assays were conducted in triplicate. The test should be repeated at least three times.

## RESULTS

**G-Ry Is a Y-1 Gene Homologue.** Full-length cDNA containing the *S. tuberosum* L. 'Golden Valley' *Ry* gene (G-Ry) and *S. tuberosum* L. 'Winter Valley' *Ry* gene (W-Ry) sequence was synthesized from mRNA, purified from 'Golden Valley' and 'Winter Valley' cultivars, respectively. G-Ry cDNA is 3570 nucleotides long, including a 168 nucleotide long untranslated region. The open reading frame (ORF) of the G-Ry was 3402 nucleotides in length and encoded for a protein of 1134 amino acids, sharing significant similarities with the R genes of the NBS-LRR class. The 24−161 region of the G-Ry amino acid harbors a domain similar to Drosophila Toll and mammalian interleukin-1 receptor (TIR) proteins (*20*). The 204−468 region of the G-Ry harbors the nuclear binding (NB) domain, and the 626−890 region harbors the LRR domain (the database search was conducted using Pfam A, B, and NCBI conserved domains). Analysis of the G-Ry sequence demonstrated 90% homology with the Y-1 of *S. tuberosum* subsp. *andigena* that was recorded in the NCBI gene bank in the DNA sequence and 83% homology in the amino acid sequence (http://www.ebi.ac.uk/Tools/clustalw2/) (**Figure 1**). The ORF of W-Ry was 3000 nucleotides in length and encoded for a protein of 1000 amino acids, sharing significant similarities with the R genes of the NBS-LRR class, in which the N-proximal region harbors a TIR domain. There was approximately 91% homology detected between G-Ry and W-Ry (data not shown) in the DNA sequence and 78% homology in the amino acid sequence (**Figure 1**).

***G-Ry* Cloning, Transformation, and Regeneration.** The full-length cDNA of *G-Ry* was synthesized from mRNA, purified from the 'Golden Valley' potato cultivar. The two fragments, 1.3 and 2.2 kb, of the *G-Ry* were PCR amplified using the primers RyF10/RyIR and RyIF/RyR10, as described in **Table 1**. Each fragment was cloned into the pUC19 cloning vector. Each of the constructs was digested with the *Xma*I and *Sph*I restriction enzymes, and the two fragments were directly cloned on the *Xma*I restriction site of the pBI121 binary vector (**Figure 2**). The cloned *G-Ry* was transformed into *A. tumefaciens* GV3101 and then cocultivated with stem segments of the 'Winter Valley' cultivar, which was the potato line that was most susceptible to $PVY^O$ infection. The internodal stem segment cuttings from the in vitro propagated 'Winter Valley' potatoes were utilized for transformation after 2 days of preculturing in stage I. The precultured explants were soaked for 10 min in infiltration medium. The explants cocultivated with *Agrobacterium* were transferred to fresh stage I medium and cultured for 3 days in darkness. After 3 days of cocultivation, they were exchanged with stage II medium including cefotaxime and kanamycin for transformant screening and then cultured for 10 days under long-day conditions. We could not detect appreciable agrobacterium growth on the stage II medium (**Table 2**). We subsequently transferred the stem segments to regeneration medium consisting of MS salts supplemented with BAP, cefotaxime, and kanamycin for organogenesis and acquired shoots from greening calli after 4−6 weeks of culturing on stage III

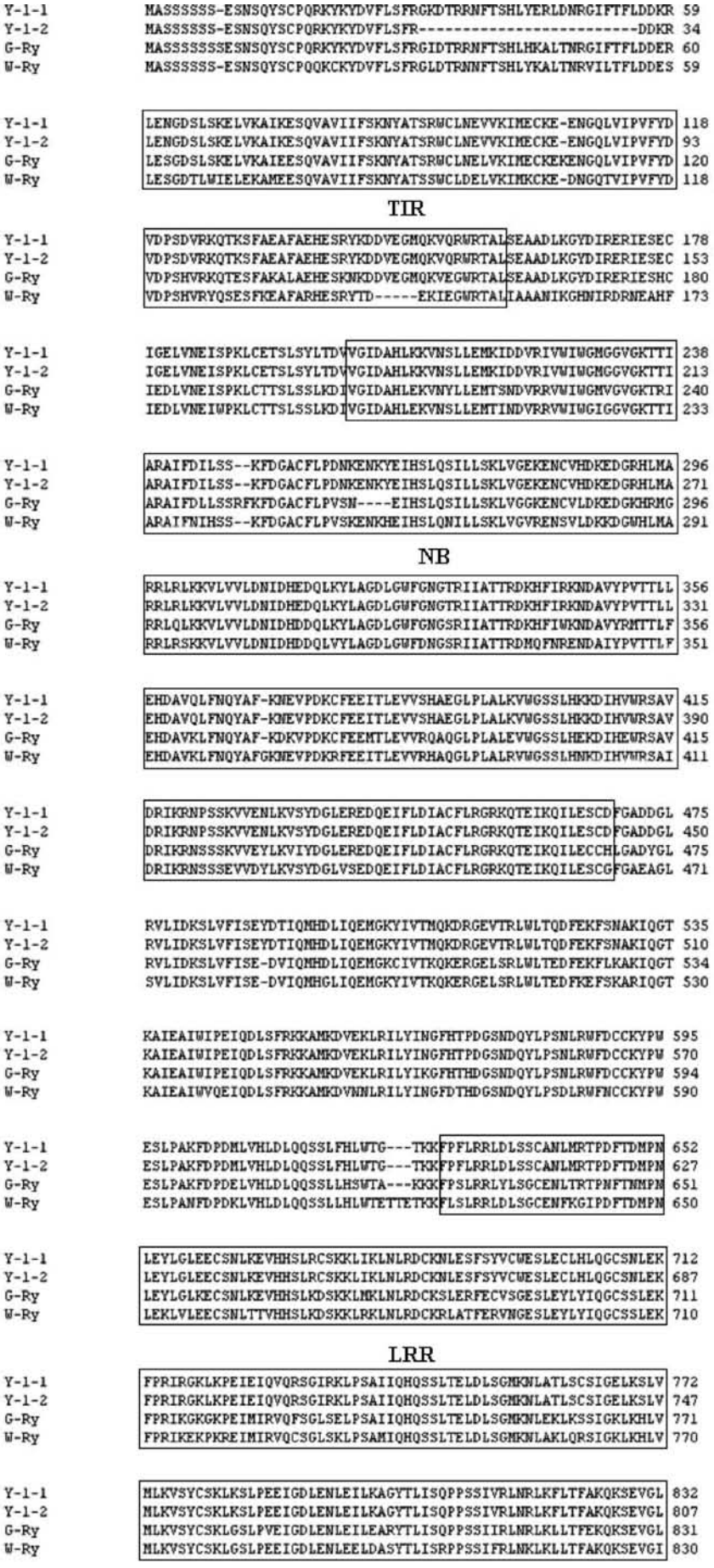


**Figure 1.** Continued

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Y-1-1          EDEVHFVFPPVNQGLCSLKTLNLSYCNLKDEGLPQDIGSLSSLEVLNLRGNNFEHLPQSL 892
Y-1-2          EDEVHFVFPPVNQGLCSLKTLNLSYCNLKDEGLPQDIGSLSSLEVLNLRGNNFEHLPQSL 867
G-Ry           EDGVHFVFPQVDGGLHSLEDLDLSYCNLIG--LPEDIDSLSSLKKLNLRGNNFEHLPQRM 889
W-Ry           KDGVHFVFPPVNRGLHSLEYLDLSYCNLIDGRLLEDIGSLSSLKVLNLRGNNFEYLPQSM 890

Y-1-1          TRLSSLQSLDLLDCKSLTQLPEFPRQLDTIYADWNNDSICNSLFQNISSFQHDICASDSL 952
Y-1-2          TRLSSLQSLDLLDCKSLTQLPEFPRQLDTIYADWNNDSICNSLFQNISSFQHDICASDSL 927
G-Ry           ARLGSLQFLNLLDCRSLTQLPELPRQLDTVYADWSNDSICKSLFQNISSFQHGICASDSL 949
W-Ry           AQLGSLQSLDLTDCKSLTQLPEFPRQLDTIYADWSNDSICKSLFQNISSFQHDICASGSL 950

Y-1-1          SLRVFTNEWKNIPRWFHHQGKDKSVSVKLPENWYVCDNFLGFAVCYSGCLIETTAQFLCD 1012
Y-1-2          SLRVFTNEWKNIPRWFHHQGKDKSVSVKLPENWYVCDNFLGFAVCYSGCLIETTAQFLCD 987
G-Ry           SLRVFTNEWKNIPRWFHHKGKGKSVSVALPENWYACDNFLGFAVCYSGCLIGTTAQLLCD 1009
W-Ry           SLRVFTNEWK-----ISQDGSTIREKIKV-------------------------YQLHCL 980

Y-1-1          EGMPCITQKLALPKHSEEFPES----------AIHFFLVPSAGLLDTSKANGKTPNDYRH 1062
Y-1-2          EGMPCITQKLALPKHSEEFPES----------AIHFFLVPSAGLLDTSKANGKTPNDYRH 1037
G-Ry           KRIPCIIQNLALCSHSEEFPESAITSSKKNPESIHFFLVPLAGLWDISKAKGKTPNDYRH 1069
W-Ry           K-------------------------------IGMHVIASWDLLYVTLVA--------- 999

Y-1-1          IMLSFSEELKEFGLRLLYRDESKLKALFKMTENNDEPTEYCVVKRRGQYDEARCSSSKKQ 1122
Y-1-2          IMLSFSEELKEFGLRLLYRDESKLKALFKMTENNDEPTEYCVVKRRGQYDEARCSSSKKQ 1097
G-Ry           MELSFSEELKEFGLRLLYKDESKLKALFQMRENNDEPTEHCIVKRRGQYDEAICSSSKKQ 1129
W-Ry           ------------------------------------------------------------

Y-1-1          RSQL 1126
Y-1-2          RSQL 1101
G-Ry           RSQL 1133
W-Ry           ----
```

**Figure 1.** Comparison of putative proteins encoded for by *G-Ry*, *Y-1* (*16*), and *W-Ry*. The overall identity of *G-Ry* and *Y-1-1* is 83% and that of *Y-1-2* is 81.2%. The N-proximal TIR domain, nucleotide binding domain (NB) region, and leucine-rich repeat (LRR) domain are boxed.

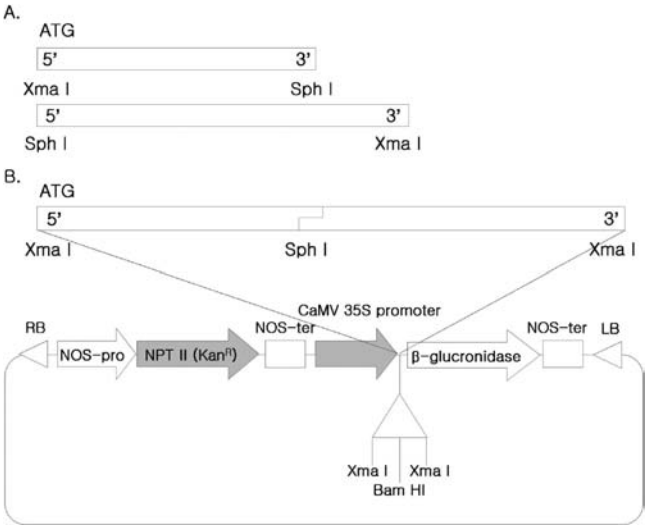


**Figure 2.** Strategy for construction: (**A**) two fragments of the *Ry* gene (left, 1.3 kb; right, 2.2 kb) were amplified by PCR; (**B**) both products were assembled on the *Sph*I linker site. pBI121 contains NPT (KanR) and CaMV promoter. In **B**, the full-length *Ry* gene was digested and cloned on the *Xma*I site of the pBI121 binary vector.

medium (**Figure 3C**,**D**). They were cultured in MS medium (nonhormone) for elongation and rooting in the presence of 100 mg/L of kanamycin, and the rooted plants were transferred to soil (**Figure 3E**).

**Expression of the *G-Ry* Gene in 'Winter Valley' Confers Resistance to PVY$^O$.** In an effort to assess *G-Ry* expression, we evaluated the transcript patterns in the 'Winter Valley'-G potatoes. Only seven lines survived in MS(Kan) medium among the 40 lines of transgenic plants examined. Seven independent transgenic plants were screened for the expression of *Ry* gene for resistance to PVY$^O$ infection in RT-PCR. Among them, two lines showed a high degree of expression (lanes 3 and 5). The highest expression lines of G-Ry were selected (lane 3). The *Ry* gene of the 'Winter Valley' potatoes did not harbor the 30 bp (base pair) at the C-terminal region as compared with the 'Golden Valley' potatoes (**Figure 1**). The primer pair including the 30 bp section was designed (**Table 1**), and RT-PCR was conducted with the primer pair. The expected band of the 138 bp fragment for the 'Winter Valley' variant and the 168 bp fragment for the 'Golden Valley' variant were present in each of the potato lines (**Figure 4A**, first and fifth lanes); 138 and 168 bp fragment amplifications were detected in the transformed 'Winter Valley' (**Figure 4A**, second and fourth lanes). As compared with the noninfected 'Winter Valley' potatoes, the transcript levels of Ry increased in the 'Winter Valley' plants infected with PVY$^O$. This result is similar to the findings of a previous study (**Figure 4A**). We confirmed *G-Ry* expression in the 'Winter Valley'-G cultivar, and our results demonstrated that *G-Ry* was expressed successfully in the 'Winter Valley'-G potatoes. Moreover, *CP* expression was not detected in the 'Winter Valley'-G plants infected with PVY$^O$ (**Figure 4B**). In the virus quantitative test using DAS-ELISA, the OD value of the 'Winter Valley'-G plants infected with PVY$^O$ was lower than that of the 'Winter Valley' plants infected with PVY$^O$ (**Figure 5**). These results indicated that G-Ry influences resistance to PVY$^O$.

**ER Resistance to PVY$^O$ Infection in *G-Ry* Expression of Transgenic Plants.** In the in vivo test, transgenic plants were multiplied using the stem propagation method and transferred to soil. 'Winter Valley' and 'Winter Valley'-G plants were inoculated with the PVY$^O$ virus at five to eight leaf stages under long-day conditions. The entire inoculation was conducted using a syringe to reduce damage to the leaf surface. The 'Winter Valley' variant was shown to be highly susceptible to PVY$^O$ infection with the development of local disease symptoms, expressed as the appearance of leaf burching and crinkling,

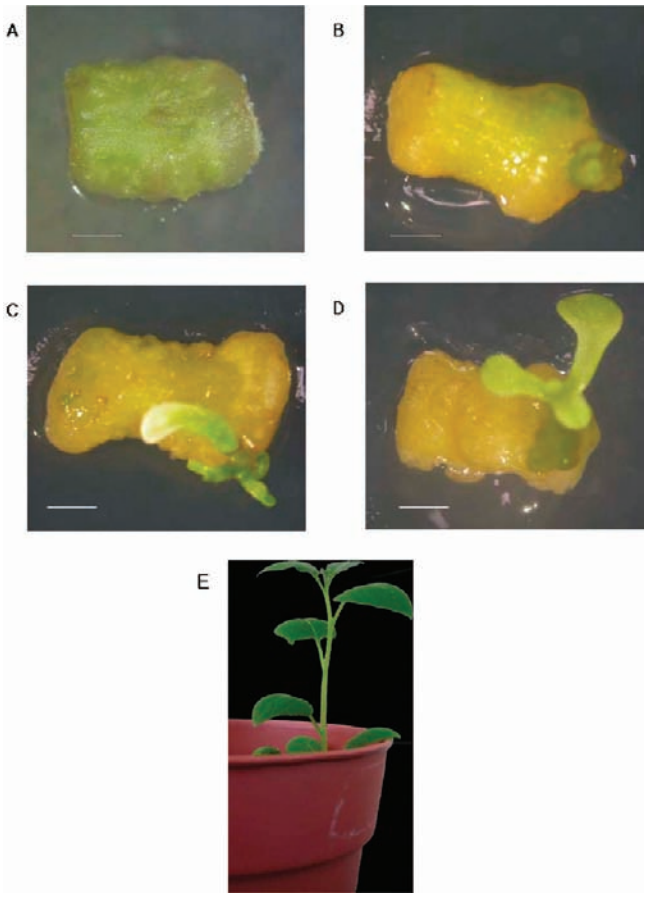


**Figure 3.** Regeneration of 'Winter Valley'-G plant: (**A**) stem segment after *Agrobacterium* infiltration on stage medium; (**B**) stem segment after *Agrobacterium* infiltration in stage medium; (**C**, **D**) development of aerial shoots in stem segment transformed by *Agrobacterium* at stage medium; (**E**) 'Winter Valley'-G grown in soil. De novo bud regeneration was obtained on calli after 4−6 weeks of incubation on stage medium. Bar = 0.2 cm (**A**−**D**).

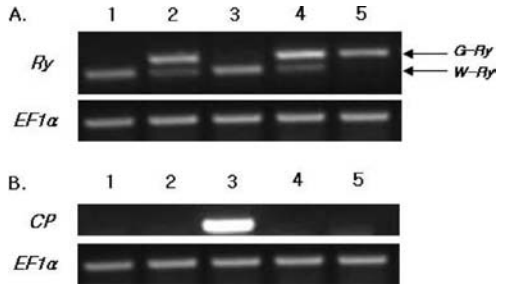


**Figure 4.** Comparative analysis of *Ry* gene and CP gene by RT-PCR: (**A**) comparative band intensities of the *Ry* gene were detected via RT-PCR using specific primers (**Table 1**) (lanes 1 and 3, 'Winter Valley'; lanes 2 and 4, transgenic 'Winter'; lane 5, 'Golden Valley'; lanes 1, 2, 5, uninfected with $PVY^O$; lanes 3 and 4, infected with $PVY^O$); (**B**) comparative band intensities of PVY coat protein (CP) gene were detected by RT-PCR using specific primers (Table**1**) (lanes 1 and 3, 'Winter Valley'; lanes 2 and 4, transgenic 'Winter'; lane 5, 'Golden Valley'; lanes 1, 2, 5, uninfected with $PVY^O$; lanes 3 and 4, infected with $PVY^O$). EF1α was used as an internal control (*32*).

mild to strong yellow leaf mosaic, necrotic veins, leaf spots, and leaf dropping (*18*). However, the infected 'Winter Valley'-G cultivar did not show any symptoms after $PVY^O$ infection, over the entire life of the plant (**Figure 6**).

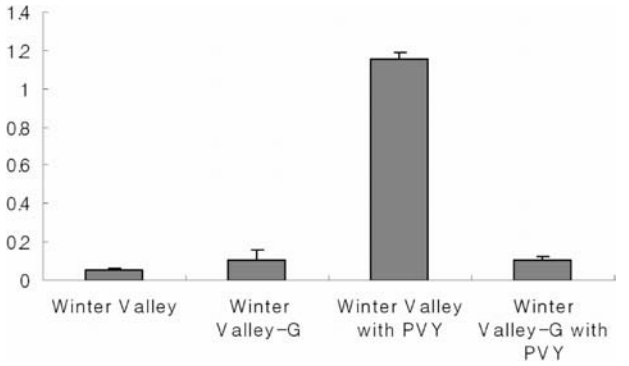


**Figure 5.** Antiviral activity of 'Winter Valley'-G against $PVY^O$ infection. DAS-ELISA ($OD_{405}$ value) was conducted at 21 days postinoculation (dpi) on the leaves above the infected leaf. The data presented herein are the average and standard deviations of three independent replicated experiments. Standard deviations are represented by error bars.

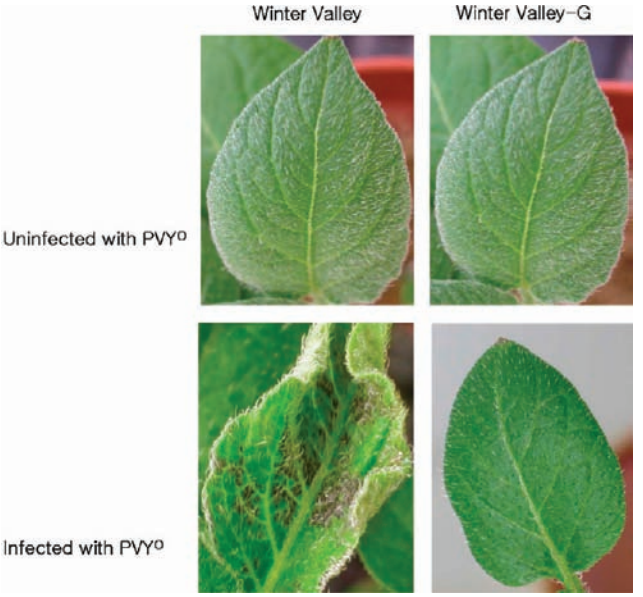


**Figure 6.** Phenotypic responses of $PVY^O$ infection. Several local lesions and leaf spots developed on most of the leaf lamina of infected 'Winter Valley' cultivars at 21 dpi. (Upper panel, uninfected with $PVY^O$; lower panel, infected with $PVY^O$).

## DISCUSSION

We reported in our earlier study that *S. tuberosum* L. 'Winter Valley' plants were highly susceptible to $PVY^O$ infection and that the 'Golden Valley' cultivar was extremely resistant to $PVY^O$ infection among Korean potato Valley cultivars (*17*). When *S. tuberosum* L. 'Golden Valley' was infected with $PVY^O$, the *Ry* gene was highly up-regulated and the CP gene could not be detected via real-time PCR and ELISA (*18*). Moreover, we also obtained sufficient results for antiviral activity from the application of the 'Golden Valley' protein and potide-G, a small (5.57 kDa) antiviral peptide isolated from the 'Golden Valley' cultivar (*18*). Therefore, we have focused on the 'Golden Valley' *Ry* gene (*G-Ry*) in an effort to improve resistance against $PVY^O$ infection for agricultural applications. To determine the antiviral activity of the *G-Ry* gene, we transformed the *G-Ry* gene into the 'Winter Valley' cultivar, which is highly susceptible to $PVY^O$ infection, and successfully obtained a 'Winter Valley'-G plant that evidenced an antiviral effect against $PVY^O$ infection (**Figures 4**−**6**). We detected 91% homology in the DNA sequence

and 78% homology in the amino acid sequence between the *G-Ry* and *W-Ry* genes. The largest class of R genes encodes a so-called nucleotide-binding site plus leucine-rich repeat (NB-LRR) type of protein. So far, all R genes that have been isolated conferring resistance to viruses belong to this class. Within the large class of NB-LRR resistance genes, a subdivision can be made on basis of the N-terminal domain. This can either be a (putative) coiled-coil (CC) domain (CC-NB-LRR), a leucine zipper domain (LZ-NB-LRR), or a so-called TIR domain, with homology to the intracellular signaling domains of the *Drosophila* Toll and the mammalian interleukin (IL)-1 recepters (TIR-NB-LRR) (*21*). The predicted protein of the *W-Ry* and *G-Ry* contained the TIR domain in its N-terminal region and the NB domain and LRR domain in the C-terminal region. Additionally, we detected a 90% homology in the DNA sequence and an 83% homology in the amino acid sequence between *G-Ry* and *Y-1* (**Figure 1**). Among the resistant (R) genes, *W-Ry*, *G-Ry*, and *Y-1* genes have been identified as members of the TIR-NB-LRR class (*22*−*25*). The *Ry* of the 'Winter Valley' cultivar also harbors the TIR-NBS-LRR region, but the 'Winter Valley' cultivar exhibited susceptible symptoms, as compared to the nonvisible symptoms of the 'Golden Valley' (*G-Ry*) and *andigena* (*Y-1*) plants against $PVY^O$ infection (*16*−*18*). The results of our current study demonstrate that the 'Winter Valley'-G plants, which expressed the *G-Ry* gene, evidenced resistance against $PVY^O$ infection (**Figures 4**−**6**), but no significant resistance to PVY infection was noted in the leaves of the transgenic potato plants expressing the *Y-1* gene under the control of the Cauliflower mosaic virus (CaMV) 35S promoter (*16*). These results indicate that structural differences in the Ry proteins or resistant pathway components such as cofactors were involved in the Ry pathway. Clearly, the resistance interaction has potential for quantitative and qualitative variations in resistance responses, depending on the elicitor−receptor interaction (*26*). For example, variable cofactors such as SGT1 (suppressor of the G2 allele of *skp1*), RAR1 (require for *Mla12* resistance), and HSP90 (heat shock protein 90) have been identified in the pathway of the resistance gene to PVX (Rx) (*27*, *28*). SGT1 positively regulates the disease resistance conferred by many R proteins, and RAR1 and HSP90 bind to SGT1 (*29*). The silencing of SGT1 in *Nicotiana benthamiana* induced a reduction in steady-state levels of the Rx (*27*). HSP90s function as cofactors of disease resistance associated with the stabilization of Rx protein levels and could be accounted for, in part, by SGT1 as well as other cofactors of disease resistance that function as cochaperones (*28*).

The results of our studies showed that *G-Ry* confers viral resistance against $PVY^O$ infection in the susceptible 'Winter Valley' cultivar when the *G-Ry* gene was expressed under the control of the CaMV 35S promoter (**Figure 6**). Improved resistance to bacterial diseases via the transformation of resistant genes was also noted in crop plants such as the tomato (*30*). *N. tabacum* cv. 'Turkish Samsun NN' plants were transformed with a modified and truncated replicase gene encoded by RNA-2 of cucumber mosaic virus strain Fny. The replicase gene had been modified by deleting a 94 bp region spanning nucleotides 1857−1950; the deletion also caused a shift in the open reading frame, resulting in a truncated translation product ≈75% as large as the full-length protein. Transgenic plants obtained were resistant to virus disease when challenged with cucumber mosaic virus virions (*31*). CP expression was readily down-regulated by the expression of *G-Ry* in the 'Winter Valley'-G cultivar (**Figure 4B**). Additionally, *G-Ry* induced extreme resistance in the virus response (**Figure 6**), and the transformation of the gene conferring resistance is a good tool for the development of virus-resistant plants.

## ACKNOWLEDGMENT

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