Molecular Screening and Characterization of Antiviral Potatoes

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Abstract Potato plants carrying the *Ry* gene are extremely resistance to a number of potyviruses, but it is not known which variety expressed the resistance. In this investigation, combined classical and molecular techniques were used to identify virus resistance potatoes. Mechanical inoculation of 32 varieties of Korean potato cultivars, with potato virus Y (PVY), induced various symptoms, such as mosaic, yellowing, necrosis, mottle, vein clearing and vein bending. Different virus spreading patterns were observed, such as highly sensitive, moderate and resistant to PVY^O inoculated leaves in different cultivars. From the results of double antibody sandwich-enzyme links immunosorbant assays (DAS-ELISA), coupled with reverse transcription polymerase chain reaction (RT-PCR), *Winter valley* and *Golden valley* were found to be highly susceptible and resistant cultivars to PVY^O, respectively. TEM was used as a complementary method to conform the localization of the virus in leaf tissues. TEM detect virus particles in *Golden valley*, where, ELISA and RT-PCR were unable to detect the *CP* gene. However, the interior part of the tissues was severely deformed in PVY^O infected *Winter valley*, than *Golden valley*. The *Ry* gene is involved in an induced response in PVY^O infected *Golden valley* plants. The methods described in this study could be applied for the screening and development of antiviral potatoes.

Keywords: potato virus Y, enzyme-links immunosorbant assays, coat protein gene, *Ry* gene, molecular screening

INTRODUCTION

Potatoes can be infected by a large number of viruses [1], and allow transfer of viruses from one vegetative generation to the next; therefore, they are also vulnerable to virus infections [2]. Potato virus Y (PVY), belonging to the virus group Potyviridae, is one of the most economically damaging plant virus that affects the potato, *Solanum tuberosum* L. PVY is transmitted by mechanical, vector or environmental means in all areas where potatoes are grown, with yield losses varying from 30 to 80% depending on the potato cultivar and virus strain [3,4]. Potato virus strains are commonly subdivided into three main strains, PVY^O, PVY^C, and PVY^N [5]. PVY^O is the common or ordinary strain in most potato producing countries. The disease is characterized by primary symptoms of necrosis, mottling and yellowing of leaves, which may cause premature death. The most widely used strategies for control of virus diseases focus on the characterization of virus resistance potato cultivars. Here, resistance is described as the effect of a virus infection being reduced or eliminated, ranging from tolerance of or hypersensitivity to the most durable extreme resistance or immunity. Few sources of extreme resistance provided by dominant genes exist for some potato viruses. An example of a durable resistance gene is a dominant gene, the *Ry* gene, which confers extreme resistance (ER) to all strains of potato virus Y in potatoes [6-8]. The *Ry* mediated resistant plants do not develop visible symptoms when challenged with the virus, the virus accumulation cannot be detected by ELISA [9-12] and the resistance is active at the protoplast level [9,13].

Korean potato cultivars vary in their sensitivity to infection with PVY^O, from highly sensitive to tolerant. Only a few potato cultivars, such as *Golden valley*, were found to be resistant to PVY^O. Different methods have been used to characterize PVY^O resistance potatoes. The most simple involve scoring the symptoms observed on PVY^O inoculated onto the first leaves of a plant. If systematic symptoms appear, it was supposed that the virus would spread from the inoculated leaves to other parts of the plant. However, this method was not applicable to all potato cultivars, as in the case of PVY^O tolerant and semi tolerant plants. Several molecular methods have been developed to identify PVY^O resistant potatoes [14-17]. Therefore, it was necessary to apply specific methods, such as molecular and serological targeting to nucleic acid and virus particles.

Here, a combined approach for the characterization of PVY^O resistant potatoes, based on ELISA and RT-PCR, is

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described. The coat protein (*CP*) gene is involved in virus spreading by cell-to-cell movement. It is possible that the *CP* gene is able to increase the SEL (size exclusion limit) of plasmodesmata and facilitate cell-to-cell movement of viral RNA. Results of ELISA and from RT-PCR of the *CP* gene were cross checked against TEM and PVY^O inoculated phenotypes, with the aim of screening PVY^O resistant potatoes from Korean cultivars. Our screening conferred that the potatoes, *Winter valley* and *Golden valley*, were highly susceptible and extremely resistant, respectively, to PVY^O. The results of this study may serve in the design and interpretation of molecular research on virus resistant plant screening and development, and facilitate the selection of resistant cultivars obtained from either classical or molecular techniques.

MATERIALS AND METHODS

Plant Materials

The 32 different varieties of virus-free Korean potato cultivars were kindly provided by the Department of Agriculture and Life Science, Kangwon University, Korea. These tubers were propagated, firstly on sterilized soil (Mix 5 Soil, Sun Grow Horticulture, Canada), and then aseptically *in vitro* via single node cutting in 3% sucrose, Murashige and Skoog (MS) medium, with plantlets obtained within 4 weeks. *In vitro* propagated plants were subcultured by transferring nodal segments to fresh medium every 4 weeks. Potato plants were grown in a growth room, at 23°C, under a 16 h photoperiod.

Virus Infection

The plant virus sample was kindly provided by the Plant Virus Genbank, Seoul Women's University, Korea. The tissue was immediately frozen in liquid nitrogen, and then finely powdered in a mortar and pestle. The powdered, frozen tissues were thawed in 1:200 (w/v) 0.1M sodium phosphate buffer (pH 7.0). Silicon carbide (400 mesh) dusted potato leaves were mechanically inoculated with prepared PVY^O inoculi. Three weeks after PVY^O inoculation, young emerging apical leaflets of each potato plant were checked for PVY^O resistance.

DAS- ELISA

The direct Double-Antibody Sandwich ELISA method (DAS-ELISA) (Agdia, Inc., Elkhart, IN, USA) was used, following the protocol provided by Agdia Inc. All reagents were obtained commercially from Agdia, Inc. Leaf samples were extracted in a 1:10 dilution of the appropriate buffer (Agdia general extract buffer), microcentrifuged at 12,000 rpm for 5 min and then added to microtiter plates. The protocol for the ELISA followed that of the manufacturer's instructions (Agdia, Inc.). The absorbance at 405 nm was determined spectrophotometrically, using an ELISA Reader (Bio-Rad, Hercules, CA, USA). All assays were performed in triplicates. Extracts of 0.1 M sodium phosphate buffer (pH 7.0) injected *Winter valley* leaves was used as a negative control. Different concentrations of PVY^O infected Burely21 was used as a positive control.

Electron Microscopy

As an alternative to ELISA, Transmission Electron Microscopy (TEM) was used for detection of the PVY^O. The quick dip technique was used, as follows. Extracted sap was microcentrifuged at 12,000 rpm for 5 min, using a table centrifuge, to remove the leaf debris. Copper grids coated with a carbon stabilized formvar film, were placed for 1 min on 20 μL of the supernatant and stained by washing the extract off with 10 drops of 2% phosphotungstic acid (PTA, pH 7.2). Coated grids were left to dry. The samples were then observed under electron microscopy (EM) (JEM-1010, JEOL Ltd., Tokyo, Japan). Appropriate pictures were taken at an acceleration voltage of 80 kV [18,19].

RT-PCR

Total RNA Extraction

Total RNA was extracted from infected leaf tissue using TRIzol reagent (Life Technologies). Leaf tissue was ground in liquid nitrogen with an autoclaved dry mortar and pestle. Total RNA was extracted from a sample of the powdered tissue (approximately 100 mg) according to the manufacturer's instructions. Total RNA was then eluted in 20 μL of RNase-free water. The RNA concentration was quantified by measuring the absorbance at 260 nm using a spectrophotometer (Bio-Rad, Smart Spec TM 3000) and gel electrophoresis on 1% agarose gel.

cDNA Synthesis

cDNA was synthesized using Superscript II reverse-transcriptase (Life Technologies). Two 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 quick chilled on ice. 5 × first strand buffer and 0.1 M DTT were added, and the mixed contents of the tube gently incubated at 42°C for 2 min. One μL (200 units) of Superscript II was added to the tube, incubated at 42°C for 50 min and the reaction stopped by heating at 70°C for 15 min. The synthesized cDNA was stored −20°C.

RT-PCR Amplification

RT-PCR amplifications were carried out on the cDNA from infected potato leaves. For amplification of the *CP* gene, the forward and reverse primers, 5′-AGGCA CATCTGGGACACATACTGTGCCGA-3′, known as YCPF, located in the Coat Protein gene position 8680–8708, according to the NCBI accession number NC_001616, and 5′-TGACTCCAAGTAGAGTATGCATACT TGGA-3′, known as YCFR, located downstream at position 9336–9365, were used. For amplification of the *Ry* gene, the forward and reverse primers, 5′-GATGGCATC ATCAT-

Table 1. The level of necrosis on the PVY^O infected leaf and those above the infection in different potato cultivars after three weeks of PVY^O infection

	PVY^O infection rate								
	Early	Golden	Gogu	Juice	Purple	Rchip	Summer	Taebuk	Winter
Infected leaf	++	–	+	++	+++	++	+	+	+++
Next leaf	++	–	++	+	++	–	–	+	+++

+++: >75% leaf area infected, ++: 50% leaf area infected, +: <25% leaf area infected, –: not infected

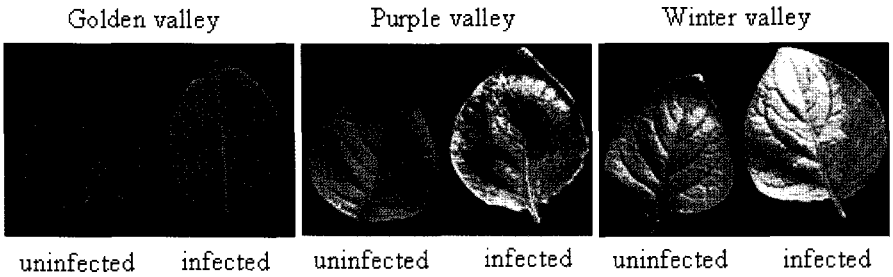

Fig. 1. All the PVY^O infected leaves (right of each variety) are those above the inoculated leaf, taken from successive positions towards the tip of *Golden valley*, *Purple valley*, and *Winter valley* plants, 21 days after inoculating the lower leaves with an extract of PVY^O. The uninfected leaf (left of each) is also same aged PVY^O uninfected plant. Several local lesions developed on the PVY^O infected *Purple valley* and *Winter valley*.

CTTCTTCTTCTGA-3′, known as Ry-1F, and 5′-CTTAG AGCTGTGACCTTTGGTTTCTTAGA-3′, known as Ry-1R, from the NCBI accession number AJ300266, were used. All primers were selected by computer analysis using the prime3 program. A 20 μL RT-PCR reaction mixture, containing 0.25 mM of dNTPs, 0.5 μM of both primers, 1 × Taq buffer, 1 μL cDNA, and 1 unit Taq DNA polymerase, was used for RT-PCR. The *CP* gene was amplified by PCR, with a denaturation of 5 min at 94°C; 40 cycles at 94°C for 30 sec; 57°C for 30 sec and 72°C for 1 min, with a final extension of 5 min at 72°C. Similarly, the *Ry* gene was amplified by PCR, with a denaturation of 3 min at 94°C; 40 cycles at 94°C for 30 sec; 52°C for 30 sec and 72°C for 7 min, with a final extension of 4 min at 72°C.

RESULTS

PVY^O Infection

PVY^O induces mild to severe mosaic, mottling or yellowing, leaf drop and premature death, and can cause stem necrosis in various cultivars. Thirty two Korean potato cultivars were inoculated with PVY^O at the six to eight leaf stage, approximately 3 week after planting. Six to seven days post-inoculation (d.p.i.), necrotic streaks along the veins of the abaxial surface of PVY^O-inoculated leaflets of susceptible plant appeared, in addition to small necrotic lesions on the interveinal region, were observed. The mosaic symptoms increased until twenty-one d.p.i. during the study period of 45 days. The leaflets of *Winter valley*, *Purple valley*, and many other varieties showed severe mosaic and crinkled symptoms, and a strong hypersensitive reaction *i.e.* systematic necrosis and some dead plants (Table 1). PVY^O infected *Golden valley* showed no symptoms of virus infection over the entire life of the plant (Fig. 1).

DAS- ELISA

Of the 32 Korean potato cultivars, the symptomatic leaves of 11, suggestive of susceptibility, mild susceptibility and resistance were selected for the ELISA test to be performed. Two leaves per plant, of a total of 5 plants per line, were inoculated therein triplicate experiments. Three weeks post-inoculation, two upper leaves from the inoculated plant were tested for PVY^O infection using DAS-ELISA. Sap of 0.1 M Sodium phosphate buffer (pH 7.0) injected *Winter valley* was used in the ELISA as negative controls. The extracts from PVY^O infected Burley21 was used in different concentrations as a positive control (Fig. 2B). The absorbance at 405 nm (optical density, OD) was measured at intervals of 20 min, and 1 and 2 h after incubation with the substrate. Data obtained from the OD readings after 2 h of incubation in the substrate were taken for further analysis. The significant differences ($P<0.05$) between the mean values was determined using the Student's *t*-test. The detection of the virus in plants tested varied according to the level of the PVY^O found in the symptomatic leaves using the ELISA test (Fig. 2A). In contrast, no PVY^O infection was detected in either the infected or non-infected leaves of *Golden valley*. This study shows that high levels of resistance to PVY^O were expressed in *Golden valley*. The OD value of the PVY^O infected *Golden valley* was found to be similar to that of the negative control (Fig. 2B). The results of this study confirm that *Golden valley* and *Winter valley* as a virus resistant and a highly susceptible, respectively, varieties of

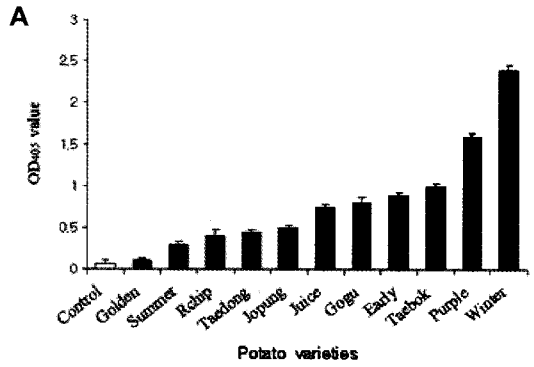

Fig. 2A. Spread of PVY^O in different cultivars, as studied with ELISA. Optical density values were obtained after 2 h of incubation in the substrate; in triplicate. Each variety is the average of five samples. Standard errors are indicated by the error bars, with significant difference at $P<0.05$. Negative Control: 21 days after injection with 0.1 M Sodium phosphate buffer (pH 7.0) in *Winter valley*, the leaf extract was used for negative control.

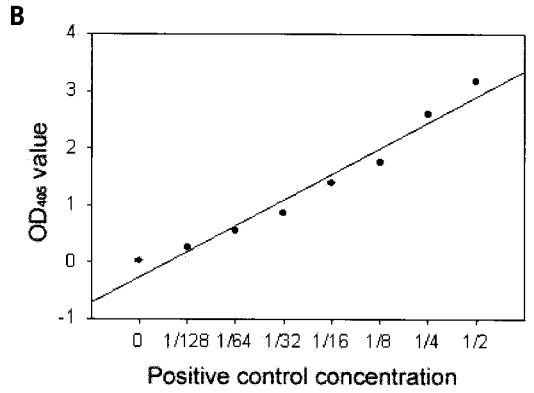

Fig. 2B. Positive Control: The sap of PVY^O infected Burley21 leaves was extracted and diluted with 0.1 M sodium phosphate buffer (pH 7.0) in different concentrations (v/v) and measured OD_{405} in ELISA Reader.

potato among those studied.

Ultra Structural Study

Transmission electron microscopy (TEM) was used mainly to clarify the ELISA results of the PVY^O infected potato leaves. Copper grids, coated with a carbon stabilised formvar film, were placed for 1 min on 20 μL of the supernatant and stained by washing the extract off with 10 drops of 2% phosphotungstic acid (PTA, pH 7.2). Samples were observed with a JEM-1010 transmission electron microscope, with an acceleration voltage of 80 k V. To observe the PVY^O in virus infected plants, negativestaining was performed. Flexuous rod-shaped virus parti cles, approximately 500 nm in length, were observed on negatively stained leaf dips of infected Burely21 (Fig. 3B). To confirm the development of PVY^O symptoms in the potatoes, sap from the leaf tissues of 3 week d.p.i. *Winter valley* and *Golden valley* plants were examined. Filamentous virus particles were observed in the samples from both the infected *Winter valley* and *Golden valley*. However, the interior part of the tissues was severely deformed in the PVY^O infected *Winter valley*, in comparison of *Golden valley* (Fig. 3D, 3F).

Expression of *CP* Gene

The coat protein (*CP*) gene is used in the assembly of virus particles and controls virus transmission by aphids. Recently, coat protein was also found to be involved in the spread of a virus by cell-to-cell movement. It is possible that the *CP* gene is able to increase the SEL (size exclusion limit) of plasmodesmata and facilitate cell-to-cell movement of viral RNA. In order to confirm the DAS-ELISA result further, the *CP* gene was detected separately in PVY^O inoculated leaves of *Winter valley* and *Golden valley* hosts using RT-PCR. The upper young leaves of 3-week post inoculated potato leaves were tested for expression of the *CP* gene. When the PVY^O coat protein region was amplified, using the two specific primers, YCPF and YCPR, a band of 690 bp in size, as expected, occurred on the 1% agarose gel of *Winter valley*; whereas, the *CP* gene could not be detected in *Golden valley* (Fig. 4). This result conferred that *Golden valley* is extremely resistant to PVY^O.

Expression of *Ry* Gene

After inoculation of the PVY^O for three weeks, young leaflets of the *Golden valley* and *Winter valley* from both inoculated and none inoculated plant leaves were harvested, the RNA extracted and cDNA synthesized. The primers, Ry-1F and Ry-1R, were used in a RT-PCR reaction. In many cultivars, no *Ry* gene could be detected (data not shown). However, a 3.4 kb band, corresponding to the expected size of the sequence included between both primers, was observed in *Golden valley*. The *Ry* gene was most strongly expressed in the PVY^O infected *Golden valley* (Fig. 5).

DISCUSSION

One of the principal threats to potato cultivation is the susceptibility of potatoes to pests and diseases. Viral diseases, in particular, cause significant qualitative and quantitative crop losses. Pre-treatment of crops with chemicals is considered the only feasible means of controlling the plant disease in agriculture fields. In recent years, interest in use of environmentally friendly alternatives to the chemical pretreatments has increased. The common strategies used for control of viral diseases focus on the molecular breeding of genetically resistant varieties. There are two major types of resistance to PVY in potatoes; extreme resistance (ER) and hypersensitive resistance (HR) [17]. A hypersensitive response is characterized by fast and localized cell death at the site of attack by

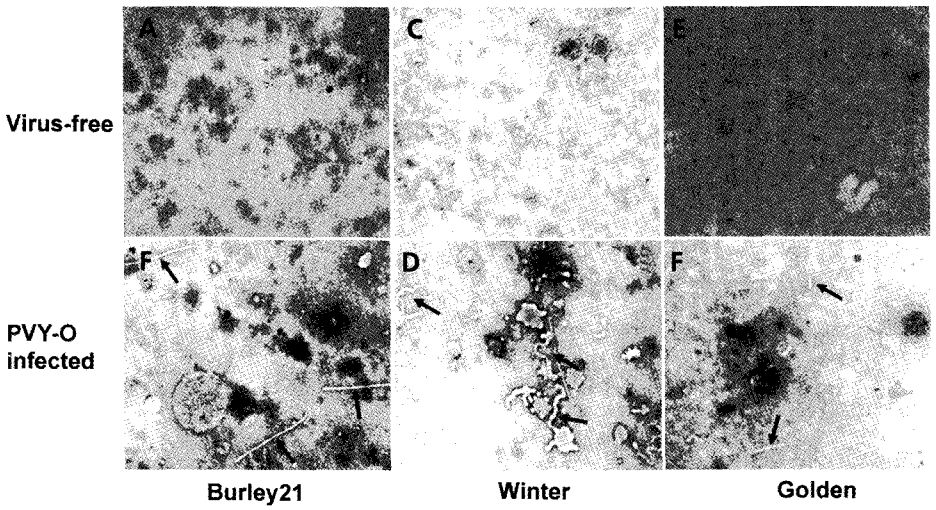

Fig. 3. Transmission electron microscopy images (80.0 kV, × 15 K) of virus-free and PVY^{O} infected Burley21, *Winter valley* and *Golden valley*. (A), (C), and (E): virus-free Burley21, *Winter valley* and *Golden valley*. (B): *Winter*-PVY^{O} Intact large virus particles. (D) and (F): *Winter*-PVY^{O} and *Golden*-PVY^{O}-scatt-ered small rod shaped virus spots. In virus infected *Winter*, compared to virus infected *Golden*, many tissues are severely deformed by infection of PVY^{O}.

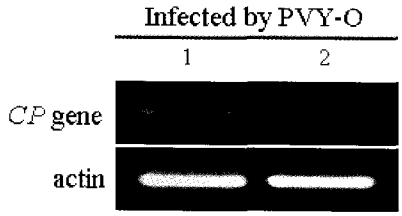

Fig. 4. Expression of the *CP* (coat protein) gene on virus-free and virus infected potato leaves, amplified by RT-PCR, using *CP* gene primers YCPF 5′-AGGCACATCTGGGACACATACTG-TGCCGA-3′ and YCPR 5′-TGACTC CAAGTAGAGTATGCA-TA-CTTGGA-3′ on total RNA from potato leaves. Lane 1: coat protein gene as expressed in PVY^{O} infected *Winter valley*. Lane 2: coat protein gene could not be detected in PVY^{O} infected *Golden valley*.

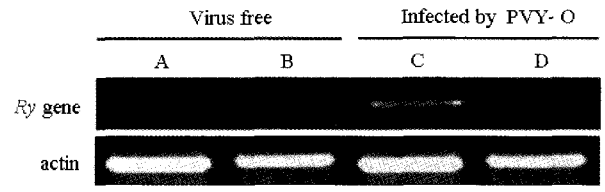

Fig. 5. Expression of the *Ry* gene in virus-free and virus infected potato leaves, amplified by RT-PCR, using primers Ry-1F (5′-GATGGCATCATCATCTTCTTC TTCTGA-3′) and Ry-1R (5′-CTTAGAGCTGTGACCTTTGGTTTCTTAGA-3′) on total RNA from potato leaves. Lane A: Virus free *Golden valley* showed weak *Ry* gene expression. Lane B: Virus free *Winter valley* did not show *Ry* gene. Lane C: After the infection with PVY^{O} Golden induced *Ry* gene and overexpressed against PVY^{O}. Lane D: After infection *Winter valley* showed no *Ry* gene expression.

pathogens in the most incompatible interactions controlled by disease resistance genes. ER strongly suppresses virus accumulation in infected cell, with no visible symptoms or detectable amounts of PVY^{O} observed in inoculated plants [6,13,21].

Few sources of extreme resistance are provided by dominant genes for some potato viruses. An example of a durable resistance gene is the *Ry* gene, which confers extreme resistance to PVY^{O}. PVY^{O} is efficiently transmitted by many aphid species in a non persistent manner. PVY^{O} infected plants exhibit mosaic, leaf crinkling symptoms, which are not readily distinguishable from veinal necrosis. In this work, 32 different Korean potato cultivars were analyzed for screening of virus resistant potatoes. The development of local disease symptoms, expressed as the appearance of local lesions, yellowing and dropping of inoculated leaves, suggestive of sensitivity to PVY^{O} occurred in many cultivars (Fig. 1). Severe local symptoms, first visible as green spots, were observed on inoculated leaves of the highly sensitive potato cultivar, such as *Winter valley*, between 5 and 7 days after inoculation. In moderately sensitive cultivars, such as *Taebok valley, Rchip valley, and Juice valley*, milder local symptoms were observed between 7 and 10 d.p.i. Syste-mic symptoms developed in most of these cultivars at the same time; between 9 and 14 days after inoculation. The infection rate of inoculated the leaf and the adjacent leaves varied according to the types of cultivar (Table 1). The cultivar *Golden valley* remained symptomless when inoculated with the PVY^{O}.

As a method for infection screening of PVY^{O} resistant varieties, it may be concluded that symptomatology is not reliable for the diagnosis of a viral disease. Detection by ELISA or other molecular techniques are necessary for the identification of PVY^{O} infection. In the context of

time and economic constraints, 21 of the 32 cultivars had to be omitted from the population for the DAS-ELISA test, as they were detected as being identical in the phenotype analysis. Variations in the symptoms of infection may be due to the cultivars used, the time of infection, the viral strain, presence of unidentified pathogens and many other factors [19]. Three weeks after PVY^O infection, the virus was detected by ELISA in eleven potato cultivars. All the samples were taken from the upper infected leaves. Later on, the virus titres in the susceptible potato cultivars reached higher levels, as detected by ELISA (Fig. 2). In *Winter valley*, the ELISA result was correlated with the appearance of local symptoms. However, this result confirms that the titre of the virus in the potato cultivars, as measured by ELISA, had different levels of sensitivity, which did not correlate with the sensitivity described by the expression of symptoms (Table 1). The ELISA method was unable to detect the virus in any of the infected *Golden valley* plants.

In order to confirm the results of the ELISA and mechanical inoculation, the presence of the *CP* gene was additionally checked in *Winter valley* and *Golden valley* using RT-PCR. The nature of the resistance response; extreme resistance or hypersensitivity, could be affected by the expression of the coat protein (*CP*) gene [23]. From the RT-PCR, *Winter valley* was shown to be highly susceptible to the PVY^O, and by comparing the DAS-ELISA and RT-PCR, as expected, the RT-PCR proved and confirmed that *Golden valley* was resistant to PVY^O.

TEM is a much less sensitive method for the detection of a virus, but could be used as a complementary method for conforming higher virus titres. In addition, it can show localization of a virus in specific tissue where the PVY^O is concentrated, as in the epidermal and phloem tissues of potato. The results from the TEM showed that virus particles were also present in *Golden valley*, although the titre was below the detection of ELISA and RT-PCR, so could not detect the *CP* gene. However, more deposition of material in the plant cells was detected in *Winter valley* by TEM in those cells that were became necrotic after inoculation with the virus. These depositions, considered part of the plant defense system, such as large numbers of mitochondria, peroxisomes and vesicles, originating from the Golgi apparatus or endo-plasmic reticulum, which requires a highly active cell metabolism [24]. The nuclei of necrotic reacting cells appeared enlarged and amoeboid (Fig. 3), which is characteristic of cells undergoing apoptosis rather than necrosis [25].

The *Ry* gene is a dominant gene, which confers extreme resistance (ER) to all strains of potato virus Y (PVY) in potatoes [26]. The molecular basis of viral resistance is not as well understood with *Ry*, although the gene has been mapped to potato chromosome XI [27-29] in a cluster of nucleotide binding site (NBS) and leucine-rich repeat (LRR) resistance gene homologous [18,30].

However, here the *Ry* mediated gene is reported to be overexpressed in plants extremely resistant against the PVY^O virus. This finding is important in understanding the resistance mechanism, as it will indicate if the *Ry* gene is involved in an induced response in infected plants (Fig. 5). However, this study suggested that the *Ry* gene confers resistance against the PVY virus. Having exploited this approach to identify the *Ry* mediated virus resistance cultivars there is a good prospect for the molecular breeding of a potato with viral resistance [31].

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REFERENCES

[1] Valkonen, J. P. T. (1994) Natural genes and mechanisms for resistance to viruses in cultivated and wild potato species (*Solanum* spp.). *Plant Breed.* 112: 1-16.

[2] Hinrichs, J., S. Berger, and J. G. Shaw (1998) A hypersensitive response-like mechanism is involved in resistance of potato plants bearing the *Ry(sto)* gene to the potyviruses potato virus Y and tobacco etch virus. *J. Gen. Virol.* 79: 167-176.

[3] Barker, H. (1993) Incidence of potato viras Y infection in seed and ware tubers of the potato cv. Record. *Ann. Appl. Biol.* 124: 179-183.

[4] Jones, D. A. C., J. A. T. Woodford, S. C. Main, D. Pallett, and H. Barker (1996) The role of volunteer potatoes in spread of potato virus YN in ware crops of cv. Record. *Ann. Appl. Biol.* 129: 471-478.

[5] De Bokx, J. A. and H. Huttinga (1981) Descriptions of plant viruses. *Commonw. Mycol. Inst. Assoc. 242.*

[6] Cockerham, G. (1943) Potato breeding for virus resistance. *Ann. Appl. Biol.* 30: 105-108.

[7] Fernandez-Northcote, E. N. (1983) Prospects for stability of resistance to potato virus Y. In *Research for the Potato in the Year 2000* (Hooker, W. J. ed.). Lima: International Potato Center. pp. 82.

[8] Ross, H. (1952) Studies on mosaic resistance in the potato. In *Proceedings of the 1951 Conference on Potato Virus Diseases*. Wageningen-Lisse, The Netherlands. pp. 40-47.

[9] Adams, S. E., R. A. C. Jones, and R. H. A. Coutts (1986) Expression of potato virus X resistant gene *Rx* in potato leaf protoplasts. *J. Gen. Virol.* 67: 2341-2345.

[10] Jones, R. A. (1990) Strain group specific and virus specific hypersensitive reactions to infection with potyviruses in potato cultivars. *Ann. Appl. Biol.* 117: 93-105.

[11] Roh, K. S. and B. Y. Choi (2004) Sucrose regulates growth and activation of rubisco in tobacco leaves *in vitro*. *Biotechnol. Bioprocess Eng.* 9: 229-235.

[12] Park, J. P., S. J. Lee, T. J. Park, K. B. Lee, I. S. Choi, S. Y. Lee, M. G. Kim, and B. H. Chung (2004) Microcontact printing of biotin for selective immobilization of streptavidin-fused proteins and SPR analysis. *Biotechnol. Bio-*

process Eng. 9: 137-142.
[13] Barker, H. and B. D. Harrison (1984) Expression of genes for resistance to potato virus Y in potato plants and protoplasts. *Ann. Appl. Biol.* 105: 539-545.
[14] Fang, Y. L., S. P. Dhital, K. H. Li, D. M. Khu, H. Y. Kim, Y. S. Song, and H. T. Lim (2005) Utilization of single nodal cuttings and therapies for eradicating double-infected potato virus (PLRV, PVY) from *in vitro* plantlets of potato (*Solanum tuberosum*). *J. Kor. Soc. Hort. Sci.* 46: 119-125.
[15] Nie, X. and R. P. Singh (2001) A novel usage of random primers for multiplex RT-PCR detection of virus and viroid in aphids, leaves, and tuvers. *J. Virol. Methods* 91: 37-49.
[16] Myslik, J. T. and A. Nassuth (2001) Rapid detection of viruses, transgenes, and mRNAs in small plant leaf samples. *Plant Mol. Biol. Rep.* 19: 329-340.
[17] Andre, N. D., C. Cesar, C. T. Antonio, and C. A. Antonio (2001) Resistance levels to two strains of *Potato virus Y* (PVY) in transgenic potatoes cv. Achat. *Hortic. Bras.* 19: 348-350.
[18] Horne, R. W. and P. Wildy (1963) Virus structure revealed by negative staining. *Adv. Virus Res.* 10: 101-170.
[19] Mori, K., R. Iriye, M. Hirata, and K. Takamizawa (2004) Quantification of *Bacillus* species in a wastewater treatment system by the molecular analyses. *Biotechnol. Bioprocess Eng.* 9: 482-489.
[20] Vidal, S., H. Cabrera, R. A. Andersson, A. Fredriksson, and J. P. Valkonen (2002) Potato gene Y-1 is an N gene homolog that confers cell death upon infection with potato virus Y. *MPMI* 15: 717-727.
[21] Hamalainen, J. H., V. A. Sorri, K. N. Watanable, C. Gebhardt, and J. P. T. Valkonen (1998) Molecular examination of a chromosome region that controls resistance to potato Y and A potyviruses in potato. *Theor. Appl. Genet.* 96: 1036-1043.
[22] Jones, J. B., J. P. Jones, R. E. Stall, and T. A. Zitter (1991) *Compendium of Tomato Diseases*. pp. 42-43. American Phytopathological Society, St. Paul, Minnesota, USA.
[23] Bendahmane, A., K. Kanyuka, and D. C. Baulcombe (1999) The Rx gene from potato controls separate virus resistance and cell death responses. *Plant Cell* 11: 781-791.
[24] Hinrichs-Berger, J., M. Harfold, S. Berger, and H. Buchenauer (1999) Cytological responses of susceptible and extremely resistant potato plants to inoculation with potato virus Y. *Physiol. Mol. Plant Pathol.* 55: 143-150.
[25] Kosslak, R. M., M. A. Chamberlin, R. G. Palmer, and B. A. Bowen (1997) Programmed cell death in root cortex of soybean root necrosis mutants. *Plant J.* 11: 729-745.
[26] Barker, H. (1996) Inheritance of resistance to potato viruses Y and A in progeny obtained from potato cultivars containing gene *Ry*: evidence for new gene for extreme resistance to PVA. *Theor. Appl. Genet.* 93: 710-716.
[27] Brigneti, G., J. Garcia-Mass, and D. C. Baulcombe (1997) Molecular mapping of potato virus Y restance gene (Ry_{sto}) in potato. *Theor. Appl. Genet.* 94: 198-203.
[28] Hamalainen, J. H., K. N. Watanable, J. P. T. Valkonen, A. Arihara, R. L. Plaisted, E. Pehu, L. Miller, and S. A. Slack (1997) Mapping and marker-assisted selection for a gene for extreme resistance potato virus Y. *Theor. Appl. Genet.* 94: 192-197.
[29] Gebhardt, C. and J. P. Valkonen (2001) Organization of genes controlling disease resistance in the potato genome. *Annu. Rev. Phytopathol.* 39: 79-102.
[30] Leister, D., A. Ballvora, F. Salamini, and C. Gebhardt (1996) A PCR based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. *Nat. Genet.* 14: 421-429.
[31] Yi, J. Y., H. W. Seo, S. W. Lee, M. S. Yang, and S. Y. Kim (2003) NPTII protein expression and resistance to bacterial disease in transgenic potato with lytic gene. *J. Kor. Soc. Hort. Sci.* 44: 187-191.

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