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Variation of *Potato virus Y* Isolated from Potato, Tobacco, Pea and Weeds in Korea on the C-terminal Region of Coat Protein Gene and 3' Non-translated Region

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***Potato virus Y* (PVY) is one of the most important viruses in many field crops in Korea. In this study, 31 PVY isolates were isolated from infected potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*), pea (*Pisum sativum*), and weeds (*Veronica persica, Lamium amplexicaule* and *Capsella bursa-pastoris*) showing different mosaic symptoms in Jeonbuk, Chungnam, Gangwon, and Gyeongbuk areas in Korea. The 640 nucleotide region containing the C-terminal portion of coat protein (CP) gene and 3' non-translated region (NTR) was amplified by reverse transcription-polymerase chain reaction (RT-PCR) using PVY-specific oligonucleotide primers. Sequence analyses of the amplified DNA fragments showed that the C-terminal portion of CP gene was not significantly different from that of previously reported PVY strains from potato (PVY-OK and -T) and tobacco (PVY-VN) in Korea. Homologies of the deduced CP amino acid sequences were 93.3-99.0% to corresponding regions of the other PVY strains including PVY^N, PVY^O, PVY^{OK}, PVY^T, and PVY^{VN}. In contrast, the sequences located at the 3'-NTR showed more diverse sequence homologies (76.4-99.7%). These results indicate that the C-terminal portion of the CP gene was relatively conserved while sequences at the 3' NTR were more diverse and variable over the host species and the regions where they were isolated.**

Keywords : *Potato virus Y*, sequence homology, subgroup, variation.

Potato virus Y (PYV) of the genus *Potyvirus* is a flexuous rod-shaped virus of ca. 750×11 nm containing a single-stranded messenger sense RNA of about 9.7 kb in length (Hinostroza-Orihuela, 1975; Makkouk and Gumpf, 1974; Robaglia et al., 1989). The genomic RNA is linked covalently to a small viral protein (VPg), polyadenylated at the 3' end, and contains a large open reading frame (ORF), which encodes 348 kDa polyprotein (de Bokx and Huttinga, 1981; Riechmann et al., 1992; Robaglia et al., 1989; Weidemann, 1988). The mature PVY structural and non-structural proteins arise by proteolytic processing of the precursor protein (Dougherty and Carrington, 1988).

PVY is an economically important plant virus causing severe diseases in tobacco, potato, pepper, and many other crops (Salazar, 1996). Recently, several PVY incidences have been reported in various potato and tobacco fields in Korea (Cheong et al., 1995; Cheong et al., 1992; Cheong et al., 1990; Cho et al., 1994; Joung et al., 1997; Park et al., 1984; Yoon et al., 1975). Since tobacco and potato fields are located near *Solanaceous* plants and weeds that harbor the virus, it is believed that PVY could be transmitted from one field to another. This may be the cause for PVY's genomic RNA variability. Although the occurrence of new PVY isolates has been reported in many countries throughout the world (Blanco-Urgoiti et al., 1998; Canto et al., 1995; Dhar et al., 1994; Hidaka et al., 1992; Inoue-Nagata et al., 2001; Liu et al., 1999; Robaglia et al., 1989; Singh and Singh, 1996; Singh et al., 1993; Sudarsono et al., 1993; Thole et al., 1993; Turpen, 1989; van den Heuvel et al., 1994; van der Vlugt et al., 1993), the three commonly recognized PVY strains namely, PVY^O (common strain), PVY^N (mild mottling strain), and PVY^C (mild mosaic and stipple-streak strain) (de Bokx and Huttinga, 1981) are still the only strains recognized and recognizable.

PVY^O causes various degrees of mosaic symptoms, whereas, PVY^N produces veinal necrosis symptoms on Burley-type tobacco cultivars. PVY^N can cause complete loss of the tobacco crop (Weidemann, 1988) and has recently been reported in Korea (Cheong et al., 1995; Cheong et al., 1992).

One approach to understand variability or virus evolution is to conduct nucleotide and amino acid sequencing of the viral genome and perform phylogenetic analysis (Rybicki and Shukla, 1992). The 3' non-translated region (NTR) and

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coat protein (CP) coding sequences have been used for the identification and classification of many plant viruses (Blanco-Urgoiti et al., 1998; Cho et al., 1994; Dhar et al., 1994; Hidaka et al., 1992; Inoue-Nagata et al., 2001; Robaglia et al., 1989; Shukla et al., 1994.; Singh and Singh, 1996; Thole et al., 1993). This study reports the nucleotide sequences of a portion of CP and the 3' NTR from 31 isolates obtained from infected tobacco, potato, pea, and weeds in Korea. The nucleotide and deduced amino acid sequences were compared with those of previously reported PVY strains, and the divergence or variance of PVY isolates was verified by conducting phylogenetic analyses.

Materials and Methods

Sources of virus isolates. Virus isolate was collected each from infected potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*), pea (*Pisum sativum*), and weeds (*Veronica persica*, *Lamium amplexicaule*, and *Capsella bursa-pastoris*) showing mild and/or severe mosaic, vein clearing and mottle in fields located at Jeonbuk, Chungnam, Gangwon, and Gyeongbuk area in Korea.

RNA extraction. Total RNAs were extracted from the infected tissues using TRIZOL (Gibco BRL, Gaithersburg, MD, USA) according to the manufacturer's protocol. The quality and relative concentrations of transcripts were checked by electrophoresis on 1% (w/v) agarose gel at 4°C and visualized by ethidium bromide staining.

RT-PCR. RT was performed on 1-2 μg of total RNA extracted from infected leaves in a reaction volume of 20 μl containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 10 mM each dNTP, 5 pmol down stream primer, and 0.5 units M-MuLV reverse transcriptase (New England

Table 1. Sources, designations, isolated locations and database accession numbers of sequences used in this study

Host species	Strain/Isolate	Isolated Location	CP amino acids[b]	3' NTR Bases	GenBank/EMBL No.
Solanum tuberosum	N	–	103	328	NC_001616
S. tuberosum	O	–	103	328	U09509
S. tuberosum	OK	na[a]	103	–	Unpubl.
S. tuberosum	T	–	103	326	D12570
Nicotiana tabacum	VN	–	103	330	U06789
S. tuberosum	KR1	Gangwon	103	328	AF264151
S. tuberosum	KR2	Gyeongbuk	103	328	AF275951
S. tuberosum	POCB1	Jeonbuk	103	328	AF424704
S. tuberosum	POCB3A	Jeonbuk	103	326	AF424705
S. tuberosum	POCB3B	Jeonbuk	103	326	AF424706
S. tuberosum	POCN6A	Chungnam	103	328	AF424707
S. tuberosum	POCN6B	Chungnam	103	328	AF424708
S. tuberosum	POCN7A	Chungnam	103	328	AF424709
S. tuberosum	POCN7B	Chungnam	103	328	AF424710
S. tuberosum	POCN7C	Chungnam	103	328	AF424711
S. tuberosum	POCN7D	Chungnam	103	328	AF424712
S. tuberosum	POKW5A	Gangwon	103	328	AF424713
S. tuberosum	POKW5B	Gangwon	103	328	AF424714
N. tabacum	TOCB1	Jeonbuk	103	328	AF424715
N. tabacum	TOCB2A	Jeonbuk	103	328	AF424716
N. tabacum	TOCB2B	Jeonbuk	103	326	AF424717
N. tabacum	TOCB2C	Jeonbuk	103	326	AF424718
N. tabacum	TOCB4A	Jeonbuk	103	328	AF424719
N. tabacum	TOCB4B	Jeonbuk	103	328	AF424720
N. tabacum	TOKW5A	Gangwon	103	328	AF424721
N. tabacum	TOKW5B	Gangwon	103	328	AF424722
Pisum sativum	WECB1	Jeonbuk	103	328	AF424723
P. sativum	WECB2	Jeonbuk	103	328	AF424724
Veronica persica	WECB3	Jeonbuk	103	328	AF424725
Lamium amplexicaule	WECBA	Jeonbuk	103	328	AF424726
Capsella bursa-pastoris	WECBB	Jeonbuk	103	328	AF424727
V. persica	WECBC	Jeonbuk	103	328	AF424728
V. persica	WECBD	Jeonbuk	103	328	AF424729
V. persica	WECBE	Jeonbuk	103	328	AF424730
V. persica	WECBF	Jeonbuk	103	328	AF424731
V. persica	WECBG	Jeonbuk	103	328	AF424732

[a] na, not available
[b] C-terminal 103 amino acids were deduced from the nucleotide sequence.

BioLabs, Beverly, USA).

PCR primers for PVY amplification were designed based on the sequence of the common strain (PVY^{O}) assembled from data in GenBank accession number U09509 (Singh and Singh, 1996). Downstream primer (PVY 3pr, 5'-GTCTCCTGATTGAAGTT-TAC-3') is complementary to nucleotides 9679 to 9698, while the upstream primer (PVY 5pr, 5'-GATGTTGCAGAAGCGTATAT-3') is located between nucleotides 9059 to 9078, giving an expected amplification product of 640 bp covering C-terminal portion of CP gene and entire 3' NTR. RT-PCR reaction was performed as described previously (Jung et al., 2000). Five μL of the amplified DNA fragments were separated by electrophoresis on a 1.0% agarose gel in 1× Tris-acetate-EDTA (TAE) buffer and stained with ethidium bromide.

Cloning and sequencing. The amplified cDNA products were ligated into TOPO cloning vector (Invitrogen Co., Carlsbad, CA, USA) as described previously (Jung et al., 2000). Recombinant plasmids containing cDNA inserts were sequenced by the dideoxynucleotide chain termination method by using the ABI prism™ Terminator Cycle Sequencing Ready Reaction Kit and an ABI Prism 377 Genetic Analyzer (Perkin Elmer, Foster City, CA, USA), located at the National Instrumentation Center for Environmental Management (CALS) of the Seoul National University, according to the manufacturer's instructions. Two independent clones of each isolate were sequenced in both directions. The partial sequences of each PVY isolate have been deposited in the GenBank (Table 1).

Phylogenetic analysis. GenBank accession numbers and sources of the virus sequences used in the alignments are shown in Table 1. Sequence analysis was carried out using the ClustalW algorithm of LaserGene™ Program (DNASTAR Inc., USA). Phylogenetic trees were constructed using the program PAUP, version 4.0 (Swofford, 1998). Phylogenies were inferred from distance matrices using parsimony (Swofford, 1998). All data sets were subjected to bootstrap analysis by performing 1000 replications and used to build a phylogenetic tree using the TreeView Win32 program.

Results and Discussion

Thirty-one isolates of PVY were collected from major tobacco and potato producing areas in Korea. RT-PCR strategy was attempted to amplify DNA fragments using specific primers designed according to the sequences of common strain (PVY^{O}) of PVY RNA. Most PCR reactions produced 640 bp DNA fragment products containing C-terminal region of CP and 3' NTR. Nucleotide sequences obtained in this study are deposited into GenBank accession numbers AF264151, AF275951, and AF424704 to AF424732. The sequences of previously reported PVY strains N, O, T, and VN were also retrieved from the GenBank database with accession numbers NC_001616, U09509, D12570 and U06789, respectively, and used for the analyses. Historically, comparative sequence analysis of these viruses has focused on the CP gene or portions thereof. This has proven to be especially useful for differentiating viruses at the species and strain levels (Shukla et al., 1994).

Sequence analysis. Two clones of each isolate were sequenced in both directions to obtain consensus sequences comprising C-terminal 103 amino acids of CP and 3' NTR. No differences in nucleotide sequences were found between independent clones representing the same PVY isolate. Table 2 shows identity percentages for CP amino acids and 3' NTR nucleotide sequences in each PVY isolate. The deduced CP amino acid sequence of each isolate is shown in Fig. 1. Consensus sequence motifs AFDF and QMKAAAL were found within the putative CP protein (Fig. 1). The homology of the CP nucleotide sequences were 91-100% while that of deduced amino acid sequence was over 95-100% in each PVY isolate. Many nucleotide variations did not result in any predicted amino acid changes, while a few variations led to some changes. In contrast, the sequences at the 3' NTR showed more variations. The nucleotide sequence identities ranged from 77.0% to 100% in each PVY isolate. These variations were also present among PVY isolates from the same hosts or regions.

Phylogenetic analysis. Phylogenetic analysis of the partial CP gene as well as 3' NTR sequences was performed using parsimony. Phylogenetic trees for the CP and 3' NTR are presented in Fig. 2. The phylogenetic trees were divided into two large subgroups on amino acid alignment of CP, N, and T types. Strain N type subgroup includes KR2, POCB1, POCN61, POCN6B, POCN7C, POCN7D, TOCB1, WECBB, WECBC, WECBD, WECBE, WECBF, WECBG, WECB1, WECB2 and WECB3, while strain T type subgroup includes KR1, POCB31, POCB3B, POKW5B, POCN7B, TOCB2A, TOCB2B, TOCB4A and TOKW5B. T type subgroup of CP branch included almost the same ratio as that of the potato- and the tobacco-infecting PVY isolates. In contrast, N type subgroup contained PVY isolates obtained mostly from potato, weeds and pea, except PVY-TOCB1 isolated from tobacco. Few amino acid variations in the putative CP protein within each subgroup were also observed (WECB1 within subgroup N, and TOCB4B, TOKW5A, TOCB2C and POCN7A within subgroup T. In general, analysis of the deduced amino acid sequences revealed that the PVY CP was highly conserved regardless of its host species and locations where they were isolated. There were only one or two substitutions at the amino acid level within the groups while a minimum of three amino acid differences were present among the groups. In contrast, analysis of the 31 sequences of the 3' NTR revealed three subgroups. The T-type subgroup of CP gene was divided into two subgroups of 3' NTR branch; one subgroup contained strain T,

Table 2. Percentage identities in deduced amino acid sequences in the C-terminal region of coat protein and nucleotide sequences of the 3' non-translated region between PVY isolates after pairwise alignment of each sequence

	N	O	OK	T	VN	KR1	KR2	POCB1	POCB3A	POCB3B	POKW5A	POKW5B	POCN6A	POCN6B	POCN7A	POCN7B	POCN7C	POCN7D	TOCB1	TOCB2A	TOCB2B	TOCB2C	TOCB4A	POCB4B	TOKW5A	TOKW5B	WECA	WECBB	WECBC	WECBD	WECBE	WECBF	WECBG	WECB1	WECB2	WECB3
CP amino acid of C terminal																																				
N		99.0	100	97.0	100	97.0	100	100	97.0	97.0	96.1	97.0	100	100	98.0	97.0	100	100	100	97.0	97.0	96.1	97.0	96.1	96.1	97.0	98.0	100	100	100	100	100	100	100	100	100
O	81.1		99.0	96.1	99.0	96.1	99.0	99.0	96.1	96.1	95.1	96.1	99.0	99.0	97.0	96.1	99.0	99.0	99.0	96.1	96.1	95.1	96.1	95.1	95.1	96.1	97.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0
OK	–	–		97.0	100	97.0	100	100	97.0	97.0	96.1	97.0	100	100	98.0	97.0	100	100	100	97.0	97.0	96.1	97.0	96.1	96.1	97.0	98.0	100	100	100	100	100	100	100	100	100
T	86.5	80.7	–		97.0	100	97.0	97.0	100	100	99.0	100	97.0	97.0	99.0	100	97.0	97.0	97.0	100	100	99.0	100	98.0	99.0	100	95.1	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0
VN	87.5	88.1	–	76.7		97.0	100	100	97.0	97.0	96.1	97.0	100	100	98.0	97.0	100	100	100	97.0	97.0	96.1	97.0	96.1	96.1	97.0	98.0	100	100	100	100	100	100	100	100	100
KR1	81.1	98.2	–	80.1	89.3		97.0	97.0	100	100	99.0	100	97.0	97.0	99.0	100	97.0	97.0	97.0	100	100	97.0	100	98.0	99.0	100	95.1	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0
KR2	80.5	98.5	–	79.4	88.7	99.1		100	97.0	97.0	96.1	97.0	100	100	98.0	97.0	100	100	100	97.0	97.0	96.1	97.0	96.1	96.1	97.0	98.0	100	100	100	100	100	100	100	100	100
POCB1	79.6	98.2	–	78.5	87.8	98.2	99.1		97.0	97.0	96.1	97.0	100	100	98.0	97.0	100	100	100	97.0	97.0	96.1	97.0	96.1	96.1	97.0	98.0	100	100	100	100	100	100	100	100	100
POCB3A	86.2	80.1	–	99.1	76.4	79.4	79.4	78.5		100	99.0	100	97.0	97.0	99.0	100	97.0	97.0	97.0	100	100	97.0	100	98.0	99.0	100	95.1	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0
POCB3B	86.8	81.0	–	99.7	77.0	80.4	79.8	78.8	99.4		99.0	100	97.0	97.0	99.0	100	97.0	97.0	97.0	100	100	97.0	100	98.0	99.0	100	95.1	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0
POKW5A	81.1	98.2	–	80.1	89.3	100	99.1	98.2	79.4	80.4		99.0	96.1	96.1	98.0	97.0	96.1	96.1	96.1	99.0	99.0	96.1	99.0	97.0	98.0	99.0	94.2	96.1	96.1	96.1	96.1	96.1	96.1	96.1	96.1
POKW5B	81.1	98.2	–	80.1	89.3	100	99.1	98.2	79.4	80.4	100		97.0	97.0	99.0	100	97.0	97.0	97.0	100	100	97.0	100	98.0	99.0	100	95.1	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0
POCN6A	79.6	98.2	–	78.5	87.8	98.2	99.1	100	78.5	78.8	98.2	98.2		100	98.0	97.0	100	100	100	97.0	97.0	96.1	97.0	96.1	96.1	97.0	98.0	100	100	100	100	100	100	100	100	100
POCN6B	78.7	97.3	–	77.3	86.9	97.3	98.2	99.1	77.3	77.6	97.3	97.3	99.1		98.0	97.0	100	100	100	97.0	97.0	96.1	97.0	96.1	96.1	97.0	98.0	100	100	100	100	100	100	100	100	100
POCN7A	81.1	98.2	–	80.1	89.3	100	99.1	98.2	79.4	80.4	100	100	98.2	97.3		99.0	96.1	96.1	96.1	99.0	99.0	96.1	99.0	97.0	98.0	99.0	96.1	98.0	98.0	98.0	98.0	98.0	98.0	98.0	98.0	98.0
POCN7B	80.5	97.9	–	79.4	89.0	99.7	98.8	97.9	78.8	79.8	99.7	99.7	97.9	97.0	99.7		97.0	97.0	97.0	100	100	97.0	100	98.0	99.0	100	95.1	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0
POCN7C	79.3	97.9	–	78.8	87.5	97.9	98.8	99.7	78.8	79.1	97.9	97.9	99.7	98.8	97.9	97.6		100	100	97.0	97.0	96.1	97.0	96.1	96.1	97.0	98.0	100	100	100	100	100	100	100	100	100
POCN7D	79.6	98.2	–	78.5	87.8	98.2	99.1	100	78.5	78.8	98.2	98.2	100	99.1	98.2	97.6	99.7		100	97.0	97.0	96.1	97.0	96.1	96.1	97.0	98.0	100	100	100	100	100	100	100	100	100
TOCB1	79.0	97.9	–	77.9	87.5	97.9	98.8	99.7	77.9	78.2	97.9	97.9	99.7	98.8	97.9	97.6	99.4	99.7		97.0	97.0	96.1	97.0	96.1	96.1	97.0	98.0	100	100	100	100	100	100	100	100	100
TOCB2A	81.1	98.2	–	80.1	89.3	100	99.1	98.2	79.4	80.4	100	100	98.2	97.3	100	99.7	97.9	98.2	97.9		100	97.0	100	98.0	99.0	100	95.1	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0
TOCB2B	86.5	80.7	–	99.4	76.7	80.1	79.4	78.5	99.1	99.7	80.1	80.1	78.5	77.3	80.1	79.4	78.8	78.5	77.9	80.1		97.0	100	98.0	99.0	100	95.1	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0
TOCB2C	86.8	81.0	–	99.7	77.0	80.4	79.8	78.8	99.4	100	80.4	80.4	78.8	77.6	80.4	79.8	79.1	78.8	78.2	80.4	99.7		99.0	97.0	98.0	99.0	94.2	96.1	96.1	96.1	96.1	96.1	96.1	96.1	96.1
TOCB4A	81.1	98.2	–	80.1	89.3	100	99.1	98.2	79.4	80.4	100	100	98.2	97.3	100	99.7	97.9	98.2	97.9	100	80.1	80.4		98.0	99.0	100	95.1	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0
TOCB4B	81.1	98.2	–	80.1	89.3	100	99.1	98.2	79.4	80.4	100	100	98.2	97.3	100	99.7	97.9	98.2	97.9	100	80.1	80.4	100		97.0	98.0	93.2	95.1	95.1	95.1	95.1	95.1	95.1	95.1	95.1
TOKW5A	81.1	98.2	–	80.1	89.3	100	99.1	98.2	79.4	80.4	100	100	98.2	97.3	100	99.7	97.9	98.2	97.9	100	80.1	80.4	100	100		99.0	94.2	96.1	96.1	96.1	96.1	96.1	96.1	96.1	96.1
TOKW5B	80.8	97.9	–	79.8	89.0	99.7	98.8	97.9	79.1	80.1	99.7	99.7	97.9	97.0	99.7	99.4	97.6	97.9	97.6	99.7	79.8	80.1	99.7	99.7	99.7		95.1	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0	97.0
WECBA	79.3	97.9	–	78.2	87.5	97.9	98.8	99.7	78.2	78.5	97.9	97.9	99.7	98.8	97.9	97.6	99.4	99.7	99.4	97.9	78.2	78.5	97.9	97.9	97.9	97.6		98.0	98.0	98.0	98.0	98.0	98.0	98.0	98.0	98.0
WECBB	79.6	98.2	–	78.5	87.8	98.2	99.1	100	78.5	78.8	98.2	98.2	100	99.1	98.2	97.9	99.7	100	99.7	98.2	78.5	78.8	98.2	98.2	98.2	97.9	99.7		100	100	100	100	100	100	100	100
WECBC	79.6	98.2	–	78.5	87.8	98.2	99.1	100	78.5	78.8	98.2	98.2	100	99.1	98.2	97.9	99.7	100	99.7	98.2	78.5	78.8	98.2	98.2	98.2	97.9	99.7	100		100	100	100	100	100	100	100
WECBD	79.6	98.2	–	78.5	87.8	98.2	99.1	100	78.5	78.8	98.2	98.2	100	99.1	98.2	97.9	99.7	100	99.7	98.2	78.5	78.8	98.2	98.2	98.2	97.9	99.7	100	100		100	100	100	100	100	100
WECBE	79.0	97.6	–	77.9	87.2	97.6	98.5	99.4	77.9	78.2	97.6	97.6	99.4	98.5	97.6	97.3	99.1	99.4	99.1	97.6	77.9	78.2	97.6	97.6	97.6	97.3	99.1	99.4	99.4	99.4		100	100	100	100	100
WECBF	79.6	98.2	–	78.5	87.8	98.2	99.1	100	78.5	78.8	98.2	98.2	100	99.1	98.2	97.9	99.7	100	99.7	98.2	78.5	78.8	98.2	98.2	98.2	97.9	99.7	100	100	100	99.4		100	100	100	100
WECBG	79.3	97.9	–	78.2	87.5	97.9	98.8	99.7	78.2	78.5	97.9	97.9	99.7	98.8	97.9	97.6	99.4	99.7	99.4	97.9	78.2	78.5	97.9	97.9	97.9	97.6	99.4	99.7	99.7	99.7	99.1	99.7		100	100	100
WECB1	79.6	98.2	–	78.5	87.8	98.2	99.1	100	78.5	78.8	98.2	98.2	100	99.1	98.2	97.9	99.7	100	99.7	98.2	78.5	78.8	98.2	98.2	98.2	97.9	99.7	100	100	100	99.4	100	99.7		100	100
WECB2	79.3	97.9	–	78.2	87.5	97.9	98.8	99.7	78.2	78.5	97.9	97.9	99.7	98.8	97.9	97.6	99.4	99.7	99.4	97.9	78.2	78.5	97.9	97.9	97.9	97.6	99.4	99.7	99.7	99.7	99.1	99.7	99.4	99.7		100
WECB3	79.6	98.2	–	78.5	87.8	98.2	99.1	100	78.5	78.8	98.2	98.2	100	99.1	98.2	97.9	99.7	100	99.7	98.2	78.5	78.8	98.2	98.2	98.2	97.9	99.7	100	100	100	99.4	100	99.7	100	99.7	
3' NTR nucleotide																																				

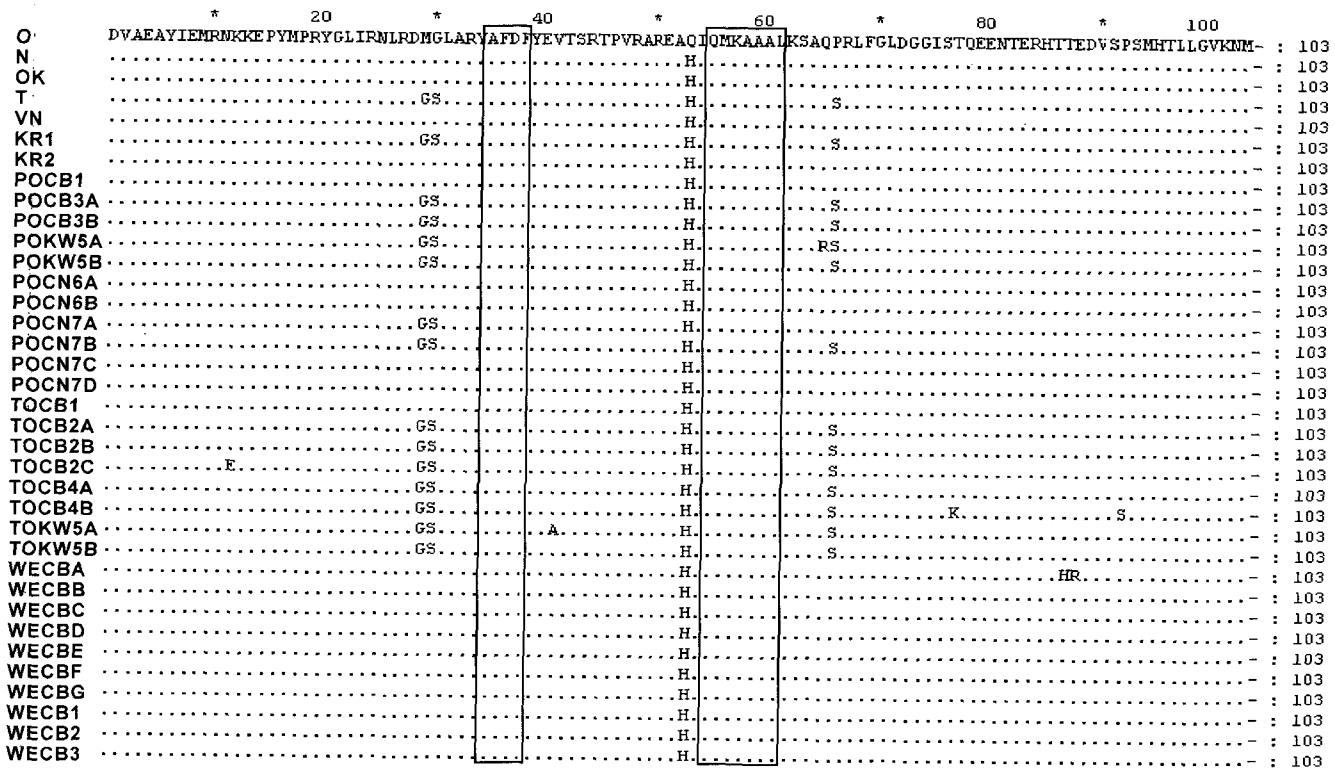

Fig. 1. Multiple alignments of the C-terminal region of coat protein (CP) amino acid of PVY isolates. The consensus AFDF and QMKAAL sequence motifs are boxed. Numbers on top represent the deduced CP amino acid position. Only the differences are shown.

POCB3A, POCB3B, TOCB2B and TOCB2C, and the other sub-group contained all of the other PVY isolates of T-type subgroup of CP gene.

Sequence variation and pathology. PVY strains infect many wild and cultivated plant species, often causing devastating diseases. PVYs infecting potato and tobacco

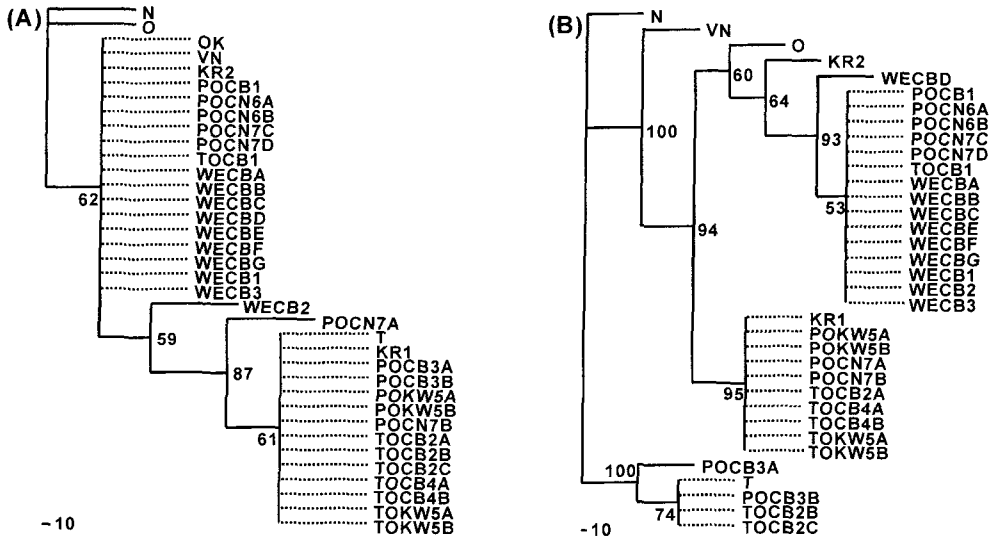

Fig. 2. Rooted phylogenetic trees derived from the multiple alignment of the amino acid sequences for the C-terminal region of CP (A) and nucleotide sequence of the 3' NTR (B) of each PVY isolate. Phylogenetic analyses were conducted by parsimony using PAUP. Statistical reliability of the nodes is obtained by bootstrap analysis (1000 replications). Numbers on branches are percentages of bootstrap analyses supporting the grouping of each branch. Bootstrap percentages less than 50% are not shown. Dotted lines within a branch represent 100% homology. The scale bar at the bottom of each panel represents the branch length of 10% divergence.

Table 3. Restriction mapping using selected enzymes and fragment sizes

RE[a]	T[b]	KR1[b]	N	O	WECB2	VN	POCB1[b]	KR2
*Taq*I	198,442	198,442	442,129,69	→	→	→	→	→[d]
*Nde*I	640	348,292	→	→	→	→	→	→
*Mbo*I	N[c]	N	522,118	574,86	→	→	→	→
*Hpa*II	N	N	N	501,139	325,315	325,176,139	→	→
*Dde*I	N	N	N	N	N	246,243,151	489,151	→
*Hpa*I	N	N	N	N	N	N	484,156	484,96,60

[a] Restriction enzymes used.
[b] PVY-T represents group of PVY isolates containing POCB3A, POCB3B, TOCP2B and TOCB2C
PVY-KR1 represents POKW5A, POKW5B, POCN7A, POCN7B, TOCB2A, TOCB4A, TOCB4B, TOKW5A and TOKW5B. POCB1 includes POCN6A to D, TOCB1, WECB (A, B, C, E, F, and G) and WECB1 to 3. Each isolate within the group showed same digestion pattern.
[c] Not required for differentiation.
[d] Arrow heads indicate same digestion patterns.

are diverse on the basis of biological properties. The 31 PVY isolates caused different symptom developments such as mosaic, necrosis, crinking, rugosity and no distinct symptom on inoculated tobacco and potato plants (data not shown). Yet, many are closely related based on nucleotide and deduced amino acid sequences of CP. These results suggest that the molecular classification using C-terminal portion of CP and 3' NTR sequences does not always explain symptom variations and is consistent with previously reported data (Chachulska et al., 1997). These results are also in agreement with recently published data showing that symptom determinants for necrosis in tobacco leaves and potato tubers are located in the HC-Pro gene and in the NIa, NIb and N-terminal portion of CP, respectively (Chachulska et al., 1997; Glais et al., 1998; Glais et al., 2002; Urcuqui-Inchima et al., 2001). Although the 31 PVY isolates reported here did not show clear differences in CP gene sequences, they generally showed more differences in the 3' NTR suggesting that there is more variation of sequences in the latter. This may be a result of the natural heterogeneity already present in a given population. In contrast, 3' NTR sequences of PVY isolates obtained from plants that are growing in adjacent fields including *P. sativum, L. amplexicaule, C. bursa-pastoris,* and *V. persica* showed a high homology (99.1-100%). This is likely an indication of either local spread of isolates from cultures to reservoir weeds or vice-versa, or the host selection pressure favoring a particular population on 3' NTR.

Sequence analysis of PVY isolates also enabled the differentiation of isolates in each branch as shown in Fig. 2. Employment of five or six restriction enzymes identified distinct PVY isolates (Table 3, data not shown). Restriction mapping can be used as a useful technique to rapidly differentiate field PVY isolates without nucleotide sequencing.

Assuming that the potato, tobacco, and weed plants used in this study are cultivated in adjacent fields in many areas in Korea, there is a high chance of virus circulation through these plants causing co-existence of different PVY isolates in the same plant. The recombination between different PVY isolates in the fields has been reported (Glais et al., 2002; Revers et al., 1996). The recombination phenomenon also causes the emergence of new isolates and, thus, increases virus variation. Sequence analysis and phylogenetic comparison conducted in this study seem to provide good indications that some of the isolates sequenced show recombination events (for example, compare the position of KR1 in the two trees in Figs. 2A and 2B). Other influences including environmental conditions that may have an effect on a particular population also increase natural heterogeneity. In addition, RNA-dependent RNA polymerases have a high degree of 'errors' that add up to more heterogeneity during virus replication. This data supports the concept of quasispecies (Domingo et al., 1985). A concern in interpreting results of this study was the possibility that differences in sequences on CP gene and 3' NTR were sometimes controlled by the error of the cloning and sequencing processes. For each experiment, at least two independent clones were sequenced and used for analysis when the same sequences were observed. While PVY will continue to evolve, it is likely that newly described or sequenced PVY isolates will fall within or expand an already existing branch of isolates. Future experiments will determine if there are more variations depending on host plants and if these amino acid and/or nucleotide substitutions between each isolate are responsible for any symptom differences and for host selections. Molecular characteristics such as pseudo-recombinants or full-length genomic RNA sequences will also be required in order to further define genes or sequences involved in host selection and symptom developments.

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