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Studies on *Potato virus Y* Isolates Infecting Potato and Tobacco in Korea

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A survey of *Potato virus Y* (PVY) was conducted in farmers' fields in 9 Korean provinces, Gyeonggi-do, Gangwon-do, Gyeongsangnam-do, Gyeongsangbuk-do, Jeollanam-do, Jeollabuk-do, Chungcheongnam-do, Chungcheongbuk-do and Jeju-do from 1998 to 2000. One hundred and fifty nine samples from potato and tobacco were collected and analyzed. Based on ELISA results, three samples PVY-tob, PVY-pot-jj and PVY-pot-chw representing PVY^N and PVY^O, respectively, were further subjected to biological, serological and molecular analysis. Host range studies showed that PVY-pot-chw is symptomatically distinguishable from PVY-tob and PVY-pot-jj isolates, both of which were symptomatically indistinguishable. However, all the three isolates failed to infect 10 capsicum varieties systemically. Isolates PVY-tob and PVY-pot-jj reacted strongly with monoclonal antibody (MAb), anti-PVY^N while PVY-pot-chw failed to react with the MAb but reacted strongly with anti-$PVY^{O/C}$. Interestingly, both PVY-tob and PVY-pot-jj isolates did not react with anti-$PVY^{O/C}$ indicating that isolate PVY-pot-chw is serologically distinguishable from both PVY-tob and PVY-pot-jj. In order to ascertain the taxonomic status of the three isolates, the 3' untranslated terminal regions (3'-UTR) were sequenced. Alignment of the sequences revealed 99% sequence identity between PVY-tob and PVY-pot-jj, both of which had 78% nt sequences identical to PVY-pot-chw. Phylogenetic analysis of the aligned 3'-UTRs clustered PVY-tob and PVY-pot-jj in the PVY^N subgroup, and PVY-pot-chw in the PVY^O subgroup.

INTRODUCTION

Potato virus Y (PVY) is a type member of the genus *Potyvirus*, family *Potyviridae* (Francki *et al.*, 1991). The virus has flexuous particles of length 740 nm containing a single-stranded messenger sense RNA genome of about 10 kb and one open reading frame (Reichmann *et al.*, 1992). The virus is transmitted by aphids in a non-persistent manner. PVY naturally infects potato, pepper, tobacco, tomato, and other solanaceous plant species causing important yield losses worldwide (de Bokx and Huttinga, 1981).

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Based on symptoms and host species, PVY isolates have been classified into three distinct groups (Y^O, Y^N and Y^C) (de Bokx and Huttinga, 1981). The PVY^O isolates (common strain) cause mottling in tobacco and crinkling, rugosity or leaf drop streaks in potato. PVY^N isolates induce necrotic symptoms in tobacco and mild mottling in potato. The PVY^C isolates cause a hypersensitive reaction in potato carrying the Nc resistance gene and induces mosaic or stipple streak in potato cultivars (de Bokx and Huttinga, 1981). In Korea, the distribution of PVY is correlated with the widespread occurrence of potato (*Solanum tuberosum* L.) and tobacco (*Nicotiana tabacum* L.). Potato and tobacco are naturally infected with PVY, with infection rates ranging from 3.7% to 12.7% (La, 1974; Lee, 1981; Park *et al.*, 1984). In potato, two PVY strains (PVY^O and PVY^N) were identified in Daegwallyeong, an alpine area, using indicator plants. Comparison of the CP sequences revealed 97.3% identity between PVY–O (Korea) and PVY–O (Japan) (Cheong *et al.*, 1992). In addition to the biological and serological data, nucleotide sequence identities of the coat protein (CP) or 3'–UTR have been increasingly used in recent years to differentiate distinct species from strains (Shukla and Ward, 1989; Frenkel *et al.*, 1989). Further, van der Vlugt *et al.* (1993) reported the subgrouping of PVY isolates based on the CP and 3'–UTR sequence identities that matched groupings based on biological and/or serological criteria. Although the occurrence of PVY on many crops is reported from South Korea, they have not been adequately characterized. In this study we report the results of a survey of PVY strains and their characterization based on biological, serological and 3'–UTR sequence properties.

MATERIALS AND METHODS

Sample collection

During May and October of 1998–2000, a survey of PVY was conducted in main potato and tobacco growing provinces of Korea namely, Gyeonggi–do, Gangwon–do, Gyeongsangnam–do, Gyeongsangbuk–do, Jeollanam–do, Jeollabuk–do, Chungcheongnam–do, Chungcheongbuk–do and Jeju–do (Table 1). The survey was conducted by walking through potato and tobacco fields, while inspecting the crops for PVY disease symptoms. Farmers in the respective fields were interviewed on the potato/tobacco variety grown, source of planting material, cultural practices and presence of other diseases. Disease incidence was calculated as the number of plants showing PVY symptoms relative to the total number of plants observed in an area.

Symptom reproduction under controlled environment

To determinate the infectivity of virus isolates and the symptoms induced on the test plant, 5–10 plant seedlings of each of the species, *Chenopodium amaranticolor*, *C. quinoa*, *Nicotiana benthamiana*, *N. tabacum* cv. 'Bright yellow', *N. tabacum* cv. 'Xanthi–nc', *N. tabacum* cv. 'Samsun', *Physalis floridana*, *Petunia* spp. at the 3–5 leaf stage were inoculated by sap extracted in 0.1 M phosphate buffer, pH 7.0. The plants were put in an insect–free greenhouse maintained at 20–25 °C with 12 h light period. Disease symptoms were recorded three times a week for 30 days.

DAC–ELISA

Because polyclonal antibodies cannot be used to differentiate PVY strains due to the high level of epitope conservation on the CPs (Shukla and Ward, 1989), a PVY specific monoclonal antibodies were used. Direct–antibody coated enzyme linked immunosorbent assay (DAC–ELISA) was conducted essentially as described by Clark and Bar–Joseph (1984) using monoclonal antibodies (MAb) purchased from Adgen (United kingdom). The MAbs and conjugate were both diluted 1:1,000 and all incubations were carried out at 37 °C for 2 h except for the substrate which was incubated for 30 min. Quantitative measures of generated *p*–nitrophenol were made by determining absorbance at 405 nm (A_{405}) in an EL312e EIA model spectrophotometer (Bio–Tek Instruments Inc., Winooski, VT, USA). Twice absorbance value was considered positive to negative control.

Electron microscopy

Two weeks after inoculation, infected leaves were harvested and immediately cut with a sharp blade (which was washed with 75% ethanol after every cut) into 1–3 mm thick pieces. The newly cut pieces were immediately fixed with 2.5% glutaraldehyde in Millonig's phosphate buffer, pH 7.0 at 4 °C. The pieces were then thoroughly rinsed in Millonig's phosphate buffer before being fixed with 2% osmium tetraoxide for 90 min. The pieces were then stained overnight in 1.0% uranylacetate at 4 °C and then rinsed in distilled water. The pieces were dehydrated six times each with 50–100% ethanol for 50 min. The dehydrated pieces were embedded in Spur resin and hardened overnight at 60 °C. Ultra–thin sections of 80 nm thickness were sliced using ultramicrotome and a knife. The sections were then stained twice, with 2% uranylacetate for 20 min then with 0.5% lead citrate for 10 min. For interpretation of results, the sections were observed under an electron microscope LEO 912AB at 80 kV.

RT–PCR

Total RNA was extracted from infected leaf samples essentially as described by Prescott and Martin (1987). The 3'–terminal region of both potato and tobacco isolates of PVY comprising part of the coat protein (CP) gene and the 3'–UTR was amplified following the RT–PCR procedure described by Pappu *et al.* (1993) using primers listed in Table 2.

Cloning and sequencing

The amplified PCR products were cloned in pGEM–T vector (Promega) and sequenced by a commercial company (Green gene biotechnology, Korea). Potato/tobacco PVY sequences obtained were phylogenetically compared to those of other PVY (GenBank and EMBL) using the multiple sequence alignment application of DNAMAN version 4.0 (Lynnon Biosoft, Quebec, Canada) full optimal sequence alignments and neighbor–joining method options of Saitou and Nei (1987) with 100 bootstrap (Felstein, 1985) replications. Percent nucleotide (nt) and ORF amino acid (aa) sequence identities between virus isolates were calculated using the distance between all pairs of sequences in the multiple alignments. PVY (CP–UTR) sequences used for comparison and their database accession numbers were as follows: AJ223592 (PVY–N854 isolate), AJ390285 (PVY–N–RB isolate), AJ390295 (PVYN–S–NTN isolate), D12570 (necrotic–PVY–T

isolate), X97895 (N605 isolate), Z70237 (Nysa isolate), U09508 (N27–92 isolate), AJ223593 (O768 isolate), D12539 (PVY–O isolate), U09509 (PVYO–Canadian isolate), AJ390289 (v942490 isolate), U10378 (Hungary pepper isolate), M22470 (N–PVY isolate), M95491 (Hungarian isolate).

RESULTS

Sample collection and analysis

A total of 139 potato and 20 tobacco leaf samples from 240 farmers' fields were collected and analyzed. Both indicator host species and DAC–ELISA revealed that 84.3% of the 159 samples were infected with PVY^N strain, 13.8% PVY^O strain and 1.9% had a mixed infection of both strains. Crosswise, 83.5% of potato samples were infected with PVY^N, 15.8% with PVY^O and 0.7% with both PVY^N and PVY^O strains. Tobacco had a higher PVY^N infection of 90.0% and only 10% of the samples were infected with PVY^O. A striking feature in tobacco was that there was no double infection of both strains (Table 1). Of all the plants tested only those in the family Solanaceae were susceptible to PVY (Table 2). In *N. tabacum*, PVY–tob and PVY–pot–jj isolates caused vein clearing and vein necrosis but PVY–pot–chw induced mild mosaic symptoms in addition to vein clearing. It is worth noting that all the three strains failed to infect and induce symptoms in *Sesamum indicum* L., *Phaseolus vulgaris* and *Datura stramonium* (Table 2). Of the 10 varieties of *Lycopersicon esculentum* tested, 6 were found to be susceptible to both PVY–pot–chw and PVY–pot–jj isolates while only 1 was susceptible to PVY–tob. However, all the 10 cultivars of *Capsicum annuum* were found to be immune to the strains as no virus was detected in the plants by either ELISA and/or electron microscopy (Table 3).

Table 1. Results of a survey of PVY in 9 provinces in Korea.

Host	Provinces	No. of detected plant [a]	PVY strain [b]		
			N+O/C	N	O/C
Potato	Gangwon-do	3	0	2	1
	Gyeonggi-do	10	0	8	2
	Gyeongsangnam-do	25	0	19	6
	Gyeongsangbuk-do	11	0	10	1
	Jeollanam-do	26	1	19	6
	Jeollabuk-do	7	0	7	0
	Chungcheongnam-do	22	0	20	2
	Chungcheongbuk-do	23	0	19	4
	Jeju-do	12	0	12	0
Tobacco	Chungcheongbuk-do	7	2	5	0
	Chungcheongnam-do	2	0	2	0
	Jeollabuk-do	11	0	11	0

[a] estimated by visual inspection
[b] estimated by ELISA

Table 2. Symptoms developed on indicator plants inoculated with PVY–pot–chw, PVY–pot–jj and PVY–tob isolates.

Indicator plant test	PVY Isolate		
	PVY–pot–chw	PVY–pot–jj	PVY–tob
Chenopodium amaranticolor	–/–(cl/–)a	–/–(cl,nl/–)	–/–(cl/–)
C. quinoa	nl/–	nl/–	nl/–
Nicotiana benthamiana	cl/cl,sM	cl/cl,sM	cl/cl,sM
N. tabacum cv. 'Bright yellow'	cl/vc,mM	nl/nl,vn	nl,vn/nl,vn
N. tabacum cv. 'Xanthi–nc'	cl/vc,mM	nl/nl,vn	nl,vn/nl,vn
N. tabacum cv. 'Samsun'	cl/vc,mM	nl/nl,vn	nl,vn/nl,vn
Physalis floridana	cl/cl,M	nl/nl,vn	cl/cl,M
Petunia spp.	cl/m	cl/vc,vn	nl/vn
Datura stramonium	–/–	–/–	–/–
Tetragonia expansa	cl/–	cl/–	cl/–
Datura stramonium	–/–	–/–	–/–
Sesamum indicum L.	–/–	–/–	–/–
Perilla frutescens	–/–	–/–	–/–
Impatiens balsamina L	–/–	–/–	–/–
Zinnia elegans Jacq.	–/–	–/–	–/–
Cucumis sativus L.	–/–	–/–	–/–
C. melo L.	–/–	–/–	–/–
Citrullus lanatus	–/–	–/–	–/–
Cucurbita moschata Duch	–/–	–/–	–/–
Raphanus sativus L.	–/–	–/–	–/–
Brassica campestris	–/–	–/–	–/–
B. rapa L.	–/–	–/–	–/–
Chrysanthemum coronarium	–/–	–/–	–/–
Glycine max Merr.	–/–	–/–	–/–
Phaseolus vulgaris L.	–/–	–/–	–/–
P. angularis	–/–	–/–	–/–
P. radiatus L.	–/–	–/–	–/–
Vicia faba	–/–	–/–	–/–
Vigna sinensis King	–/–	–/–	–/–

[a] cl, chlorotic local; nl, necrotic local; mM, mild mosaic; m, mosaic; sM, severe mosaic; vc, vein clearing; vn, vein necrosis; inoculated leaves/upper leaves; –, no symptoms.

Serological relationships

Both PVY–tob and PVY–pot–jj isolates reacted strongly with anti– PVY^N serum but failed to react with anti–$PVY^{o/c}$ while PVY–pot–chw isolate reacted strongly with anti–$PVY^{o/c}$ but failed to react with anti– PVY^N. Therefore PVY–tob and PVY–pot–jj are serologically distinguishable from PVY–pot–chw.

Electron microscopy

Examination of the thin sections from the infected potato and tobacco revealed typical cytoplasmic inclusions bodies like pinwheels, scrolls, laminated aggregates and tubes in cells of *N. tabacum* cv. 'Xanthi–nc' (Fig. 1). Additionally, many virus particles were spotted around the mitochondria. Crude sap observation revealed long flexuous particles of 750 nm in length, but no inclusion bodies.

Table 3. Infectivity of PVY–pot–chw, PVY–pot–jj and PVY–tob isolates in tomato and red pepper cultivars.

Indicator plant test		PVY Isolate		
		PVY–pot–Chw	PVY–pot–Jj	PVY–tob
Lycopersicon esculentum cv	'Dotaerang'	+[a]	+	+
	'Dotaerang B'	+	+	–
	'House dotaerang'	+	+	–
	'Yeonggwang'	+	+	–
	'Seogwang'	+	+	–
	'Ponterosa'	+	–	–
	'Youngmuja'	–	+	–
	'Minicarol'	–	–	–
	'PePe'	–	–	–
	'Yoyo'	–	–	–
Capsicum annuum cv	'Bugwang'	–	–	–
	'Dongbang'	–	–	–
	'Geosung'	–	–	–
	'Pungchon'	–	–	–
	'Gumtop'	–	–	–
	'Daewon'	–	–	–
	'Daemung'	–	–	–
	'Joyang'	–	–	–
	'Chungok'	–	–	–
	'Umsung'	–	–	–

[a] +, symptoms; –, no symptoms.

Table 4. Primers used for amplification of the 3'–terminal untranslated region (3'–UTR) of PVY isolates from Korea.

Primer	Sequence [a]	Primer Position
HRP 1	5'TTT TTT TTT TTT TTT TTT TTT A3'	poly A tail
HRP 2	5'TTT TTT TTT TTT TTT TTT TTT G3'	poly A tail
HRP 3	5'TTT TTT TTT TTT TTT TTT TTT C3'	poly A tail
HRP 4	5'TGG TGY ATH GAN AAT GG3'	CP (conserved WCIEN region)

[a] Primers designed by Pappu *et al.* (1993); N=A, C, G, T; Y=C, T; H=A, T, C.

3'–UTR sequence comparison with known PVY isolates

The PVY–pot–chw's 3'–UTR sequenced was found to contain 720 nucleotides (nts) including part of the CP of 150 amino acids, while isolates PVY–tob and PVY–pot–jj each contained 325 nts. A detailed analysis of the aligned 3'–UTR nucleotide sequences revealed a varying degree of sequence identity with isolates PVY–tob and PVY–pot–jj sharing 99% nt and only 89% identity with isolate PVY–pot–chw. A pair–wise comparison

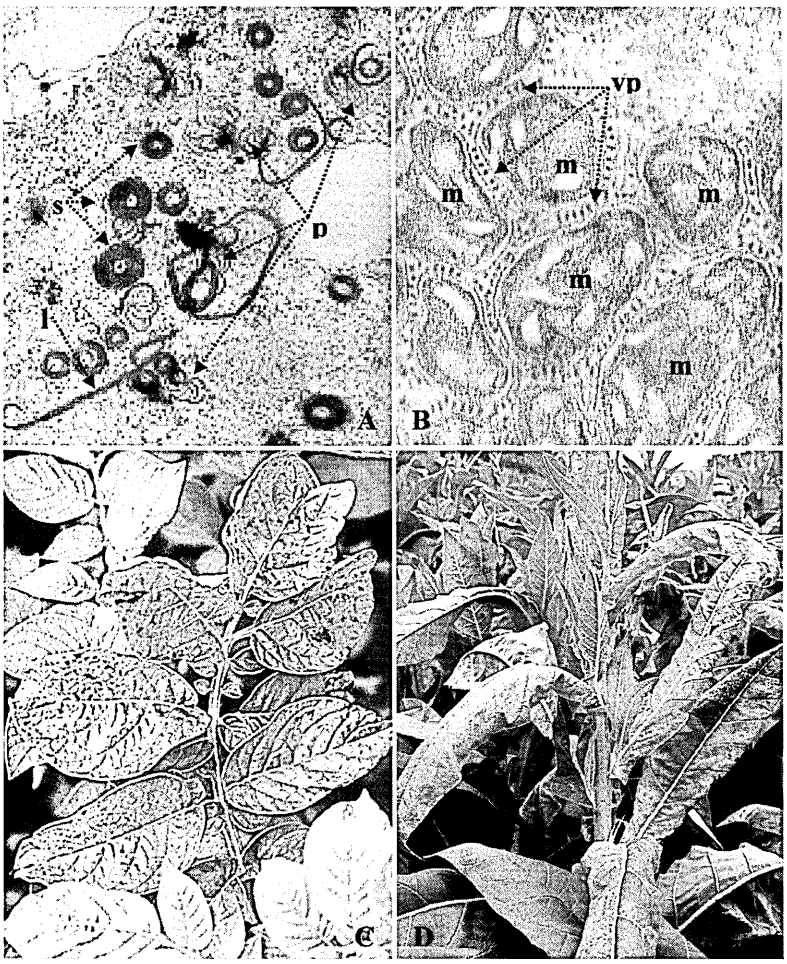

Fig. 1. Typical cytoplasmic inclusions bodies like pinwheels, scrolls, laminated aggregates and tubes (A) and virus particles around the mitochondria (B) in cells of *Nicotiana tabacum* cv. 'Xanthi-nc'. Potato plants (C) showing necrotic symptoms by PVY-pot-jj isolate and tobacco plants (D) showing vein clearing and necrotic symptoms caused by PVY-tob isolate in the fields. m, mitochondria; vp, virus particles; s, scrolls; l, laminate aggregates; p, pinwheels.

of the 3'-UTR sequences of PVY-tob, PVY-pot-jj, and PVY-pot-chw isolates with those of the other PVY isolates from the database revealed 76–99% sequence identity (data not shown). Phylogenetic analysis of the aligned sequences revealed a tree structure whose clusters defined separate groups with both PVY-pot-jj and PVY-tob isolates falling within the PVY^N group while potato-chw fell within the PVY^O group (Fig. 2).

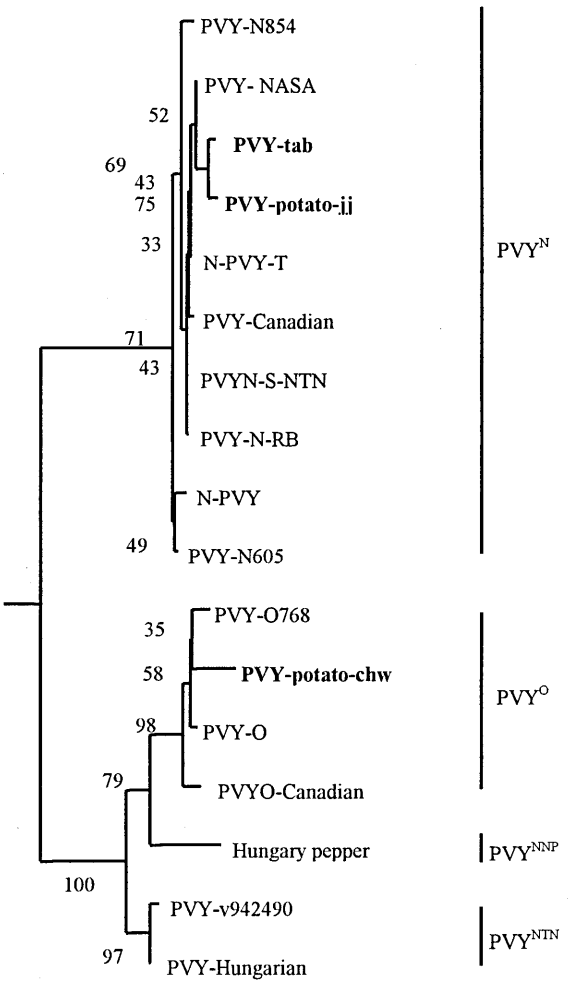

Fig. 2. Phylogenetic tree constructed from nucleotide sequence alignments of the 3'–UTR fragments of PVY isolates from tobacco and potato.

DISCUSSION

Symptoms of PVY–pot–jj and PVY–tob such as necrotic spots, vein and petiole necrosis indicated that the isolates belonged to the necrotic strains group, while PVY–pot–chw isolate that exhibited vein clearing and mild mosaic belonged to the common strain group. An interesting observation is that all isolates failed to infect *C. annuum*, although it contradicts the finding of Lee (1981) that PVY infects *C. annuum*.

Nevertheless, 6 of the 10 varieties of *L. esculentum* tested were infected with both PVY–pot–chw and PVY–pot–jj while PVY–tob infected only one variety of *L. esculentum*. To reveal the exact identity of these isolates, their 3'–UTRs were sequenced and compared with those of 15 distinct PVY isolates. High degree of 3'–UTR sequence identities (84–99%) of both PVY–pot–jj and PVY–tob isolates with known PVY isolates confirmed them as strains of PVY as they were within the cut–off range of 83–99% for identifying strains of the same virus (Frenkel *et al.*, 1989). The 3'–UTR sequences of PVY–pot–jj and PVY–tob isolates differed from those of known tobacco vein necrotic strains of PVY occurring in Korea (PVY–VN). This possibly indicates the existence of variation in the necrotic isolates of PVY within the country. The 3'–UTR has been shown to regulate symptom severity (Rodriguez–Cerezo *et al.*, 1991). Further studies involving additional PVY isolates representing different agro–climatic regions of the country are required in order to detect and identify the existence of various distinct PVY isolates. This would help in developing an effective strategy for managing the viruses.

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