Three Strain Groups of Potato A Potyvirus Based on Hypersensitive Responses in Potato, Serological Properties, and Coat Protein Sequences

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ABSTRACT

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Isolates of potato A potyvirus (PVA) were obtained from Michigan (PVA-U), Maine (PVA-M), and Hungary (PVA-B11; complete nucleotide sequence known). PVA-U caused systemic necrosis in potato (*Solanum tuberosum*) cv. King Edward, which carries the gene *Na* for hypersensitivity to PVA, whereas PVA-M caused mottle symptoms without necrosis. In other potato cultivars carrying *Na*, PVA-U and PVA-M caused systemic necrosis. Therefore, the hypersensitivity gene in King Edward was different from *Na* and was named Na_{KE}. PVA-B11 caused no hypersensitive response and infection in potato cultivars except for cv. Pito, which was infected but showed no symptoms. All PVA isolates caused necrotic local lesions on detached leaves of A6 (*Solanum demissum* × *S. tuberosum* cv. Aquila). Thus, PVA-U, PVA-M, and PVA-B11 represented distinct strain groups of PVA, which were named PVA-1, PVA-2, and PVA-3, respectively. Determination of the coat protein (CP) nucleotide sequence of PVA-U and PVA-M and comparison with the CP of PVA-B11 revealed 16 amino acid changes among the three PVA isolates. Six changes that affected the amino acid properties (charge and/or hydrophobicity) distinguished PVA-B11 from PVA-U and PVA-M and were located at the immunodominant N-terminus.

Potato A potyvirus (PVA) belongs to the large and agriculturally important group of potyviruses (18). The host range of PVA is limited to the family Solanaceae. PVA can be transmitted mechanically, but it is usually field-transmitted by aphids in a nonpersistent manner in the field. PVA occurs in potato (*Solanum tuberosum* L.) worldwide, but it is not very well studied compared to other important potato-infecting viruses such as potato Y potyvirus (PVY), potato leafroll luteovirus (PLRV), potato X potexvirus (PVX), and potato M and S carlaviruses (PVM and PVS) (2,19,24).

Flexuous filamentous particles of PVA are ca. 730 nm long and 15 nm in diameter and are relatively labile in purified preparations (16). PVA is moderately immunogenic and serologically related to PVY, the type member of the potyvirus group (2,16,18). The single-stranded RNA genome of PVA is 9,565 nucleotides long and contains one open reading frame encoding a polyprotein of 3,059 amino acids. The genome structure of PVA is similar to other potyviruses sequenced so far (15,32). The PVA coat protein (CP) is 269 amino acids long and has significant amino acid sequence homology (73 to 78%) to the CPs of other potyviruses (31,32).

Typically, a continuum of isolates exists within a potyvirus species, with overlapping host ranges and varying levels of serological relationships (4,17,37). The N-terminus of the viral CP is exposed to the surface of virions and is the immunodominant region, carrying epitopes specific to an individual virus isolate rather than to the virus group (36). It is therefore expected that sequence differences within the CP-coding region, particularly at the N-terminus, are reflected as differences in serological properties between isolates of a potyvirus such as PVA.

Diseases observed in potatoes (*Solanum tuberosum* L.) in Germany and in Maine in 1911 and 1912 (28) were probably the first reported ones attributable to PVA, which was described and named almost 20 years later (27). Later criteria such as the physical properties and symptoms in potato and other test plants were used to identify distinct strains of PVA from North America and Europe (24). Several strains of PVA, identified on the basis of a wide variation in symptom expression, were observed to occur simultaneously in a single potato crop, cv. Ulster Torch, in the field (6). Symptoms in infected potatoes are usually mild, but yield losses up to 40% have been observed (2). Coinfection with PVA and PVX may cause a more severe crinkle disease (24,27).

Some potato cultivars and wild species react with top necrosis, i.e., with a hypersensitive response, to infection with PVA (8,24,34). Extreme resistance to PVA, defined as a low incidence of infection in intact plants and an extremely low virus titer in infected plants, is also known to exist in potato species (10,39). Hypersensitivity and extreme resistance to PVA in potato are controlled by single dominant genes. All genes for extreme resistance to PVA and certain, but not all, genes for hypersensitivity to PVA, are also functional against PVY (8,10,21,24,34).

Virus isolates can be grouped according to their ability to elicit a certain hypersensitivity gene or genes in potato, which is the basis of the strain group concepts for PVX (9) and PVY (21). However, PVA isolates have not been systematically classified into strain groups on a similar basis. The strain group concept is useful for the determination of locally prevalent virus isolates and for prediction of cultivar responses in the field, and it assists breeding for additional virus resistance (40). Furthermore, nucleotide sequence analysis for significant differences and production of infective cDNA clones of virus isolates (5) representing different strain groups may reveal the viral gene products interacting with plant resistance genes. This is important, because different plants appear to have evolved to recognize different gene products of viruses (13).

In this study, we characterized the hypersensitive reactions in potatoes, serological properties, and CP sequence homology of one European isolate and two American isolates of PVA. The isolates were found to be different for most, but not all, of the properties studied. Importantly, they differed in their elicitation of a hypersensitive resistance response in potato and therefore are considered to represent three distinct strain groups of PVA.

MATERIALS AND METHODS

Plants. Pathogen-tested in vitro plantlets of the potato cvs. Gladstone, Green Mountain, Irish Cobbler, Jemseg, and Maris Piper, and the potato clone A6 (*Solanum demissum* Lindl. × *S. tuberosum* cv. Aquila) were obtained from the Cornell

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Uihlein Foundation Seed Potato Farm. Pathogen-tested in vitro plantlets of the potato cvs. King Edward and Pentland Ivory were obtained from the Department of Plant Production, University of Helsinki, Finland. Plants were transferred to soil and were grown under natural daylight in an air-conditioned greenhouse from March to September. The daily means of the minimum and maximum temperatures were 18°C and 22°C.

Virus isolates. PVA-U was originally isolated from the Michigan potato breeding line M700-14 (40) and was obtained from the collection at the Department of Plant Pathology, Cornell University. PVA-M was isolated from potato cv. BelRus in Maine, and it was kindly provided by F. Mehdizadegan, Maine Department of Agriculture. PVA-B11 from Hungary has been propagated in *Nicotiana clevelandii* Gray and was kindly provided by F. Rabenstein, Federal Center for Plant Breeding Research, Aschersleben, Germany. The plant species from which PVA-B11 was originally isolated is not known. These isolates were known to be of PVA because they reacted serologically with polyclonal antibodies to PVA but not with polyclonal antibodies to PVY, and because infected tobacco plants (*Nicotiana tabacum* L. cv. Samsun NN) did not respond hypersensitively, and thus these isolates did not belong to the tobacco veinal necrosis strain group of PVY (PVY^N; 19). PVA-U and PVA-M infected potato cv. Pentland Ivory, causing no hypersensitive response, and thus belonged neither to strains C and O of PVY nor to potato V potyvirus (21). The entire RNA sequence of PVA-B11 has been determined (31,32). PVA-U and PVA-M were propagated in potato cv. Jemseg (extremely resistant to PVX and hypersensitive to PVY^O; 40) and PVA-B11 was propagated in tobacco cv. Samsun in the greenhouse.

Inoculation. For virus experiments, potato plants were multiplied by taking shoot cuttings in the greenhouse. Plants were mechanically inoculated by grinding PVA-infected leaves with a mortar and pestle at 1 g per 5 ml of distilled water and rubbing the sap onto the two largest leaves of each potato plant dusted with Carborundum (each plant had a total of six leaves). Experiments with mechanical inoculation were carried out twice. For graft-inoculation, one apical shoot (2 to 3 cm) of a virus-infected plant (for PVA-U and PVA-M, potato cv. Jemseg; for PVA-B11, tobacco cv. Samsun NN) was side-grafted onto a 1-week-old potato plant as previously described (39). Vigorous growth of the grafted scion was taken as an indication of a successful graft union. Scions were not removed. Graft-inoculation experiments were carried out three times.

The A6 local lesion assay was carried out by mechanically inoculating PVA onto Carborundum-dusted detached leaves of A6 (a local lesion host for PVA and PVY) using the dry inoculation method (14). Inoculated leaves were placed on wet filter papers in petri dishes, which were enclosed in transparent plastic bags and incubated at 22°C under a 16-h photoperiod (140 $\mu E\ s^{-1}\ m^{-2}$).

Virus detection. Polyclonal antibodies and alkaline phosphatase conjugated monoclonal (Agdia, IN) or polyclonal antibodies to PVA (Boehringer Mannheim, Germany) were used for virus detection by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (7). None of these antibodies reacted with PVY. Uppermost fully expanded leaves were sampled, weighed, and ground in ELISA extraction buffer at 1 g per 3 ml. Two aliquots (100 µl) were transferred into two wells of a microtiter plate (Corning, NY, or Greiner Labortechnik, Frickenhausen, Germany). Color reactions were developed using *p*-nitrophenyl phosphate as a substrate, and absorbances were recorded at 405 nm using a microplate reader (Titertek Multiscan or BIO-TEK Automated Microplate Reader EL311).

Comparison of serological properties. Correlation between PVA accumulation and ELISA absorbance values, i.e., serological reactivity, was examined among the three PVA isolates 16 days after graft-inoculation. The A6 local lesion assay and DAS-ELISA were performed in parallel from the same leaf sample. In three experiments, two plants each of potato cvs. Jemseg and Pito and tobacco cv. Samsun were sap-inoculated, and tests were carried out 18 days after inoculation.

Western analysis. Leaves of potato cv. Pito infected systemically with one of each of the three PVA isolates (15 days after graft-inoculation) were ground with a mortar and pestle in ELISA extraction buffer (phosphate-buffered saline [PBS]: 136 mM NaCl, 1.5 mM KH2PO4, 20 mM Na2HPO4, 2.7 mM KCl (pH = 7.4), 2% polyvinyl pyrrolidone, 0.2% bovine albumin). Extracts were filtered through two layers of Miracloth (Calbiochem-Behring, La Jolla, CA) into test tubes and were stored at 24oC. Aliquots (10 ml) of sap were collected for Western analysis at 0, 18, 28, 46, and 65 h after extraction and were stored at –20oC. All samples were analyzed in parallel. The AP-conjugated antibodies to PVA used in Western analysis were polyclonal (Boehringer) and were diluted following the supplier's instructions. Western analysis was carried out as previously described using X-phosphate and nitro blue tetrazolium as a substrate (38).

Immunoelectron microscopy (ISEM). ISEM was carried out as previously described (33). Sap was extracted from PVA-infected potato cv. Pito and was diluted 10-fold with 0.06 M phosphate buffer (pH = 6.5) containing 0.05 M EDTA. Carbon-coated grids coated with PVA antibodies were floated on drops of the diluted sap. Virions were observed using a Jeol 100 CX electron microscope (Jeol, Ltd., Tokyo, Japan).

Reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA used for PCR was isolated from leaves of infected plants by phenol-chloroform extraction following a previously described procedure (42). Two oligonucleotide primers of 21 and 20 bases flanking the PVA CP gene sequence were used, namely GTACTGAACTGGAAAAGTACT (primer 1; upstream) and CCCTGACAGTTGAAACATAA (primer 2; downstream) (32). Total

Table 1. Symptoms in systemically infected potato and tobacco following graft-inoculation and in detached leaves of A6 following mechanical inoculation with three isolates of PVA

	Symptoms[a]			Genes for resistance to potyviruses	
	PVA-U	PVA-M	PVA-B11	Genes[b]	References
Potato cultivars					
Gladstone	NS	NS	NI	*Na:ny*	8
Irish Cobbler	NS	NS	NI	*Na*	24
Maris Piper	NS	NS	NI	*Na:Nv:Nc:ny*	21
King Edward	NS, R, W	Mo, R, W	NI	Na_{KE}*:Nv:Nc:ny*	21; This study
Allegany	SS	SS	NI	*na:Ny*	40
Jemseg	Mo	MM	NI	*na:Ny*	40
Pentland Ivory	SS	SS	NI	*na:Nv:nc:Ny*	21
Pito	Motr, W	Motr, W	SS	*na*	This study
Hybrid A6[c]	NLL	NLL	NLL	Ny_{DMS}	10
Tobacco					
cv. Samsun	MMo	MMo	MMo	*Hr*	19

[a] Systemically infected leaves: MM, mild mosaic; MMo, mild mottle; Mo, mottle; Motr, mottle expressed transiently; NS, necrotic spots; R, reddish brown pigmentation; SS, symptomless systemic infection detected by enzyme-linked immunosorbent assay (ELISA); W, wavy leaf margins; NI, no detectable infection by ELISA at 16, 21, and 28 days after inoculation. Mechanically inoculated leaves: NLL, necrotic local lesions.

[b] Genes for specific hypersensitivity to the following potyviruses and potyvirus strains: *Na*, PVA strain groups 1 and 2; Na_{KE}, PVA strain group 1; *Nc*, PVY strain group C; *Ny*, PVY strain group O; Ny_{DMS}, PVY and PVA; *Nv*, PVV; Hr, tobacco veinal necrosis strain group of PVY.

[c] *Solanum demissum* × *S. tuberosum* cv. Aquila.

RNA was denaturated at 65°C for 5 min. Reverse transcription was performed in a reaction mix of 10 μl containing 10 mM Tris-HCl (pH = 8.3), 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.01 M gelatin, 1 mM of each dNTP, 20 pmol primer 2, 20 U RNasin (Promega, Madison, WI), and Moloney murine leukemia virus reverse transcriptase (Gibco BRL). The reaction mix was incubated at 42°C for 1 h and was combined with 40 μl of PCR mix containing 10 mM Tris-HCl (pH = 8.3), 50 mM KCl, 0.01% gelatin, 20 pmol primer 1, and 1 U *Taq* polymerase (Perkin-Elmer Corp., Norwalk, CT). A drop of mineral oil was layered onto the reaction mix. Amplification was carried out using the following cycle: initial denaturation at 95°C for 5 min, and then 35 cycles of annealing (48°C, 1 min), primer extension (72°C, 1 min), and denaturation (95°C, 1 min).

Cloning and sequencing of amplified fragments. Amplified DNA fragments were separated by electrophoresis in an agarose gel and isolated from the gel using Wizard PCR Preps DNA Purification System (Promega). Purified DNA fragments were cloned using pGEM-T Vectors System (Promega) following the supplier's instructions. Three independent cDNA clones of CP of each PVA isolate were selected for sequence analysis and were restriction-mapped and subcloned. Both strands of subclones were sequenced by the dideoxy chain-termination method (35).

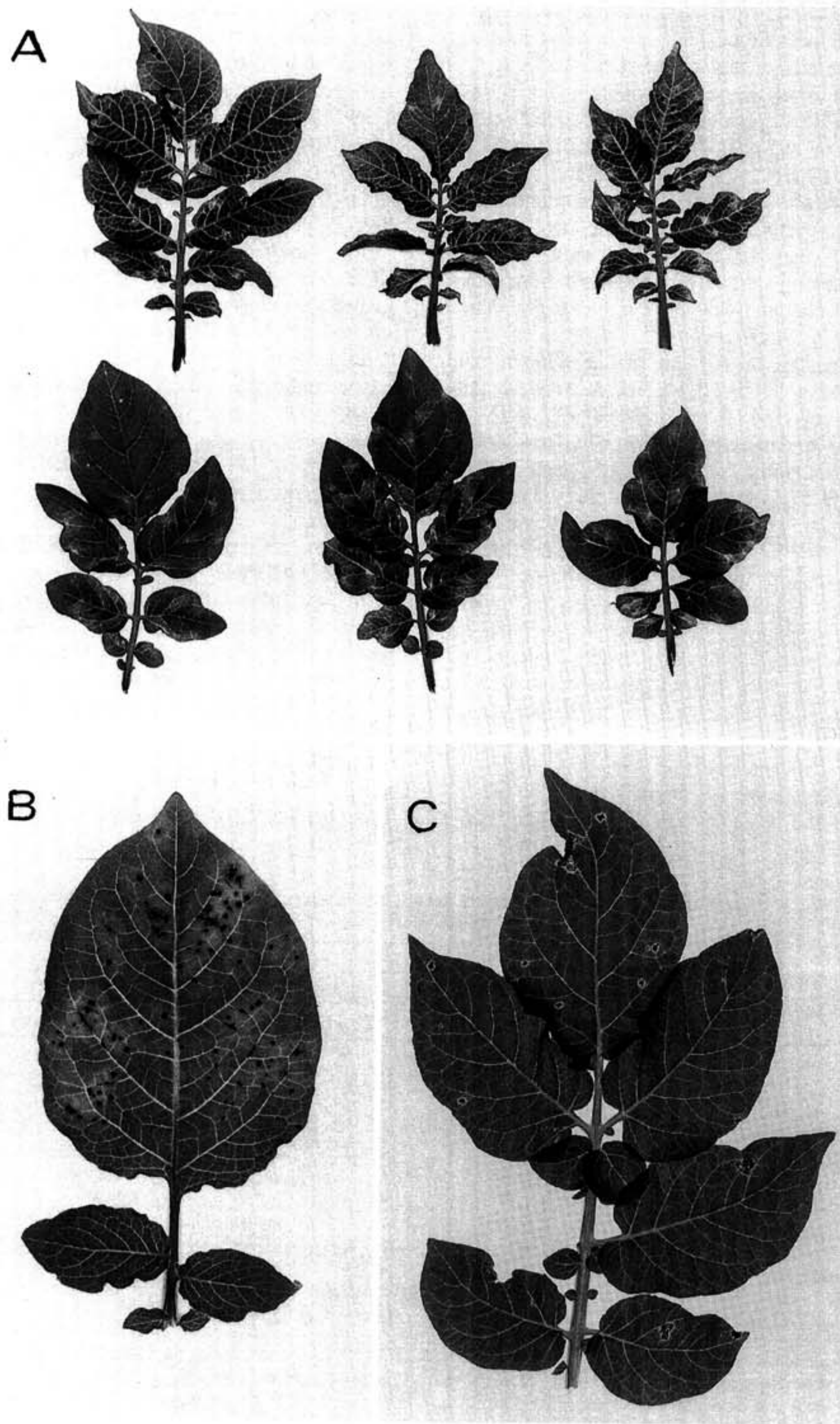

Fig. 1. (A) Systemically infected leaves of potato cv. King Edward 21 days after graft-inoculation. Upper row, necrotic lesions caused by potato A potyvirus isolate U(PVA-U); lower row, mottle symptoms caused by PVA-M. **(B)** Necrotic lesions on a detached leaf of A6 4 days after dry-inoculation with PVA-M. **(C)** Necrotic lesions on the systemically infected leaves of Maris Piper 21 days after graft-inoculation with PVA-M.

RESULTS

Hypersensitive responses in inoculated potato plants. PVA-U and PVA-M caused numerous necrotic lesions on the systemically infected leaves of potato cvs. Gladstone, Irish Cobbler, and Maris Piper 15 to 17 days after graft-inoculation (Table 1). Both PVA isolates caused similar numbers of lesions, but those on Gladstone were larger than those on the other two cultivars. No top necrosis was observed. On the systemically infected leaves of King Edward, PVA-U caused circular chlorotic lesions in which the center turned necrotic 2 days after the appearance of the lesion (Fig. 1). In contrast, PVA-M caused mottle symptoms that were severe on the axillary shoots of King Edward, and no necrosis was observed (Fig. 1). PVA-U and PVA-M caused reddish pigmentation at leaf margins of the systemically infected leaves of King Edward, and the leaflets developed a wavy symptom; titers of PVA-U and PVA-M were similar according to ELISA (0.79 ± 0.27 and 0.77 ± 0.13 for PVA-U and PVA-M, respectively).

PVA-U caused mottle symptoms and mild chlorosis; whereas PVA-M caused mild mosaic on the leaves of Jemseg. Titers of PVA-U and PVA-M were similar according to ELISA and the A6 local lesion tests (Table 2). Symptoms became masked and the titers of PVA-U and PVA-M decreased as greenhouse temperatures occasionally exceeded 22°C; virus titers recovered 2 days after the greenhouse became cooler. PVA-U and PVA-M accumulated similar titers in cv. Pito (Table 2), causing transient mottle symptoms on leaves 14 to 17 days after graft-inoculation (symptoms were visible for 3 to 4 days), and the leaves remained wavy (Table 1). Virus titers remained constant in infected Jemseg and Pito maintained in the greenhouse under standard conditions and tested by ELISA at 3, 5, and 8 weeks after inoculation (data not shown). Allegany and Pentland Ivory were infected systemically with PVA-U and PVA-M but developed no symptoms (Table 1).

PVA-B11 caused no systemic infection in graft-inoculated plants of Gladstone,

Irish Cobbler, Maris Piper, King Edward, Allegany, Jemseg, and Pentland Ivory according to ELISA and the A6 local lesion assay (Table 1). Pito became systemically infected with PVA-B11, but no symptoms were observed (Tables 1 and 2). PVA-B11 was detected in Pito by ELISA at 14 and 21 days after inoculation, but not at 30 days after inoculation. Apical shoots from healthy plants of Pito were grafted onto the tops of previously graft-inoculated Jemseg, but no shoot of Pito was PVA-infected 20 days after grafting, according to ELISA. No local lesions appeared on the mechanically inoculated leaves of Gladstone, Irish Cobbler, King Edward, Maris Piper, and Pentland Ivory, and no systemic infection was detected by ELISA 21 days after inoculation. Noninoculated (systemically infected) leaves of nine Pito plants of the 32 mechanically inoculated with PVA-B11 in three experiments had high ELISA readings (1.57 ± 0.33 for infected Pito; 0.04 ± 0.02 for noninoculated Pito) at 16 and 21 days after inoculation; no Pito plant had ELISA readings different from those of the noninoculated plants at 30 days after inoculation.

Comparison of serological properties using A6 local lesion assay and ELISA. All three isolates of PVA produced necrotic lesions on detached leaves of A6. Lesions caused by PVA-M (Fig. 1) and PVA-U were visible 3 days after inoculation and were morphologically similar; whereas lesions caused by PVA-B11 were visible 5 days after inoculation and were larger. Numbers of lesions caused by all three PVA isolates were similar following inoculation from infected tobacco (Table 2). ELISA readings for PVA-B11 were significantly higher than for PVA-U and PVA-M, however. The numbers of lesions caused by PVA-U and PVA-M inoculated from Pito were significantly higher than those of PVA-B11, while the ELISA readings for PVA-B11 were higher than those for PVA-U and PVA-M. Numerous lesions were observed on A6 leaves inoculated from leaves of Jemseg previously graft-inoculated with PVA-U or PVA-M, and ELISA readings for both PVA isolates were positive. In contrast, no lesions were produced on the A6 leaves inoculated from Jemseg previously graft-inoculated with PVA-B11, and ELISA was negative (Table 2).

Properties of PVA particles. ISEM revealed similar particles of ca. 730 nm in the sap of Pito infected with each PVA isolate. Western analysis suggested that the CP of PVA-B11 was relatively stable, because significant degradation was not observed until 65 h. The CPs of PVA-U and PVA-M were intact at the beginning of the experiment, but they degraded gradually with time. However, significant portions of PVA-U and PVA-M CP core were still intact at 65 h (data not shown).

The CP amino acid sequences. The amino acid sequence deduced from the nucleotide sequence of the CP gene of PVA isolates M (EMBL accession z49088) and U (EMBL accession z49087) and their sequence comparison with the CPs of PVA-B11 (31,32) is shown in Figure 2. Of the 16 amino acid differences among the three isolates, 10 differences were located at the N-terminal region of the CP. More importantly, six of these 10 differences led to a change in the properties of the amino acids (charge and/or hydrophobicity) and were located at the N-terminal region

Table 2. Assessment of potato A potyvirus (PVA) titers by the A6 local lesion assay and double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) in systemically infected leaves of tobacco and potato 18 days after mechanical inoculation[a]

	PVA-U		PVA-M		PVA-B11	
	Lesion (no.)	A_{405}	Lesions (no.)	A_{405}	Lesions (no.)	A_{405}
A6 assay[b]						
Potato cv. Jemseg	112±32		96±22		0	
Potato cv. Pito	96±16		103±20		13±7	
Tobacco cv. Samsun	31±17		28±12		37±30	
ELISA[c]						
Potato cv. Jemseg		0.42±0.08		0.45±0.10		0.04±0.020
Potato cv. Pito		0.34±0.06		0.26±0.09		1.15±0.14
Tobacco cv. Samsun		0.32±0.11		0.24±0.06		1.53±0.07

[a] The same uppermost fully expanded leaf from each test plant was analyzed by both methods in parallel. Presented numbers are means for ELISA absorbance values (A_{405}) and numbers of necrotic lesions (±standard error of the mean) generated by two plants of each variety in three experiments.

[b] Each value is a mean number of lesions on the total of 24 A6 leaves. No lesions developed on A6 leaves mock-inoculated using leaves from healthy plants. Least significant difference = 21 ($P < 0.05$).

[c] The sap extracted from potato cvs. Jemseg and Pito was diluted 2.5×10^{-1}. Each value is a mean of the total of 12 ELISA readings. $A_{405} = 0.04 \pm 0.03$ for healthy noninoculated plants. Least significant difference = 0.16 ($P < 0.05$).

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             1                                                     50
  pva-B11    AeTLDAsEal AQKsEGrqKE GEsNSgkAVA VKDKDVDLGT AGTHSVPRLK
  pva-CAN    AgTLDAgEtp AQKsEGkkKE GEgNSgkAVA VKDKDVDLGT AGTHSVPRLK
    pva-M    AgTLDAgEtp AQKcEGkkKE GEgNSgkAVA VKDKDVDLGT AGTHSVPRLK
    pva-U    AgTLDAgEtp AQKsEGrkKE GEgNSsrAVA VKDKDVDLGT AGTHSVPRLK
Consensus    A-TLDA-E-- AQK-EG--KE GE-NS--AVA VKDKDVDLGT AGTHSVPRLK

             51                                                   100
  pva-B11    SMTSKLTLPM LKGKSVVNLD HLLSYKPKQV DLSNARATHE QFQNWYDGVM
  pva-CAN    SMTSKLTLPM LKGKSVVNLD HLLSYKPKQV DLSNARATHE QFQNWYDGVM
    pva-M    SMTSKLTLPM LKGKSVVNLD HLLSYKPKQV DLSNARATHE QFQNWYDGVM
    pva-U    SMTSKLTLPM LKGKSVVNLD HLLSYKPKQV DLSNARATHE QFQNWYDGVM
Consensus    SMTSKLTLPM LKGKSVVNLD HLLSYKPKQV DLSNARATHE QFQNWYDGVM

             101                                                  150
  pva-B11    ASYELEESSM EIILNGFMVW CIENGTSPDI NGVWTMMDnE EQVSYPLKPM
  pva-CAN    ASYELEESSM EIILNGFMVW CIENGTSPDI NGVWTMMDnE EQVSYPLKPM
    pva-M    ASYELEESSM EIILNGFMVW CIENGTSPDI NGVWTMMDnE EQVSYPLKPM
    pva-U    ASYELEESSM EIILNGFMVW CIENGTSPDI NGVWTMMDgE EQVSYPLKPM
Consensus    ASYELEESSM EIILNGFMVW CIENGTSPDI NGVWTMMD-E EQVSYPLKPM

             151                                                  200
  pva-B11    LDHAKPSLRQ IMRHFsAlaE AYIEMRSREK PYMPRYGLQR NLRDQSLARY
  pva-CAN    LDHAKPSLRQ IMRHFsAlaE AYIEMRSREK PYMPRYGLQR NLRDQSLARY
    pva-M    LDHAKPSLRQ IMRHFsAlaE AYIEMRSREK PYMPRYGLQR NLRDQSLARY
    pva-U    LDHAKPSLRQ IMRHFkAtrE AYIEMRSREK PYMPRYGLQR NLRDQSLARY
Consensus    LDHAKPSLRQ IMRHF-A--E AYIEMRSREK PYMPRYGLQR NLRDQSLARY

             201                                                  250
  pva-B11    AFDFYEITAT TPiRAKEAHL QMKAAALKNS NTNMFGLDGN VTTSEEDTER
  pva-CAN    AFDFYEITAT TPvRAKEAHL QMKAAALKNS NTNMFGLDGN VTTSEEDTER
    pva-M    AFDFYEITAT TPvRAKEAHL QMKAAALKNS NTNMFGLDGN VTTSEEDTER
    pva-U    AFDFYEITAT TPiRAKEAHL QMKAAALKNS NTNMFGLDGN VTTSEEDTER
Consensus    AFDFYEITAT TP-RAKEAHL QMKAAALKNS NTNMFGLDGN VTTSEEDTER

             251              269
  pva-B11    HTATDVNRNM HHLLGVKGv
  pva-CAN    HTATDVNRNM HHLLGVKGv
    pva-M    HTATDVNRNM HHLLGVKGl
    pva-U    HTATDVNRNM HHLLGVKGv
Consensus    HTATDVNRNM HHLLGVKG-
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Fig. 2. Amino acid sequence comparison of the coat proteins of potato A potyvirus (PVA) isolates PVA-B11, PVA-CAN, PVA-M, and PVA-U. The predicted amino acid sequence is presented in the single letter code, and the differences are indicated in bold letters. The lowest row represents the consensus sequence where differences are indicated by a broken line.

(E2G, S7G, A9T, L10P, G18K, and S23G) (Fig. 2). Interestingly, these six amino acids were similar in PVA-U and PVA-M, which thus distinguished the two from PVA-B11. For the remaining four differences at the N-terminal region of the CP, the amino acids at positions 14 and 17 were identical for PVA-B11 and PVA-U but different from PVA-M; whereas the amino acids at positions 26 and 27 were identical for PVA-B11 and PVA-M but different from PVA-U. PVA-U differed from the other two isolates at amino acid positions 139, 166, 168, 169, and 260. Differences at amino acid positions 166, 168, and 169 may be important, since there is a significant change in the charge and/or hydrophobicity of the amino acid.

The CP sequences of PVA-U and PVA-M were compared with the previously published CP sequence of PVA-CAN from North America (11). The CPs of PVA-M and PVA-CAN were similar except for two amino acid differences (C14S and L269V), which did not affect the properties of the amino acids (Fig. 2).

DISCUSSION

Responses in potato cultivars following inoculation suggested that all three PVA isolates were different. The major difference between PVA-U and PVA-M was that PVA-U caused a hypersensitive response in King Edward but PVA-M did not. Responses in other potato cultivars to these two isolates were similar. PVA-B11 caused no detectable infection in any potato cultivar except Pito, in which no symptoms were observed. All PVA isolates caused necrotic lesions on the leaves of A6, which is hypersensitive to several viruses, such as PVA, PVY, and some isolates of PVX, tobacco mosaic tobamovirus (TMV) (10,14), and cucumber mosaic cucumovirus (41). Based on these results, we postulate that PVA-U represents PVA strain group 1 (PVA-1), which includes isolates eliciting the hypersensitivity gene *Na* previously described in potato cultivars (10,21,24). PVA-M is postulated to represent PVA strain group 2 (PVA-2), which is defined by the ability to infect King Edward without triggering the hypersensitive response. Existence of the PVA-2 group suggests that the gene for hypersensitivity to PVA in King Edward differs from those of other cultivars, which are elicited by both PVA-1 and PVA-2. Therefore, we propose the name Na_{KE} for the gene in King Edward. PVA-B11 is postulated to represent PVA strain group 3 (PVA-3), which includes isolates that elicit no hypersensitive response in potato cultivars carrying the gene *Na* or Na_{KE}. The observation that PVA isolates may differ in their elicitation of a resistance response in potato cultivars is novel. Distinguishing PVA-U, PVA-M, and PVA-B11 as strains is consistent with many of the criteria used for recognition of virus strains (25) and the historical usage for PVX and PVY (9,21). Because the plant species from which PVA-B11 was originally isolated is not known, it remains to be shown whether strain group 3 isolates of PVA naturally infect potatoes in the field.

The absorbance values for PVA-U and PVA-M were low in ELISA, even though the high numbers of local lesions on A6 leaves indicated that virus titers were high in test plants. In contrast, PVA-B11 had high ELISA absorbance values, but the numbers of local lesions on A6 leaves were similar to or lower than those of PVA-U and PVA-M. These differences among the three PVA isolates may be explained by a higher reactivity of the PVA-B11 CP with the antibodies, or by less effective elicitation of hypersensitivity in A6 by PVA-B11.

Comparison of the amino acid sequence of PVA-B11 CP with PVA-U and PVA-M revealed 16 amino acid differences. Of these changes, 10 were located at the N-terminus, four in the middle region, and two in the C-terminal region of the CP. From the 10 amino acid changes at the N-terminal region, six changes led to altered amino acid property (charge and/or hydrophobicity) and could also affect the immunological properties. PVA-B11 differs at these amino acid positions from PVA-U and PVA-M, and also from PVA-CAN from Canada (10). Clustering of the amino acid differences at the N-terminal region of PVA CPs is consistent with our results, which suggest that PVA-B11 differs from PVA-U and PVA-M for serological reactivity because the N-terminus is the immunodominant region of PVA (and potyvirus CPs in general) (1,36).

An interesting subject for further studies will be to determine which genomic areas of the three PVA isolates are responsible for the differences in resistance responses in potato observed in this study. The viruses most widely studied regarding their interactions with plant resistance genes, and which naturally infect potato, are PVX and TMV (19). Certain potato cultivars carry the genes *Nx* and/or *Nb,* which control strain specific hypersensitive responses to PVX strain group 3 (PVX-3) and 2 (PVX-2), respectively; and both genes are also elicited by PVX strain group 1 (9,20). Certain potato cultivars carry the gene *Rx* for comprehensive extreme resistance to PVX strain groups 1, 2, and 3 (10,34). The CP gene of PVX-3 is the avirulence gene corresponding to *Nx*; whereas the CP gene of PVX-2 is not responsible for induction of *Nb* (3). The CP gene of PVX, however, is the avirulence gene corresponding to *Rx* and is an important determinant of viral fitness in potato (3). The CP genes of TMV and tomato mosaic tobamovirus (ToMV), closely related to TMV, are the avirulence genes corresponding to the *N′* gene controlling hypersensitive response to these viruses in *Nicotiana sylvestris* Speg. (12,22,30). Mutation of the TMV CP gene may also be responsible for the induction of bright yellow mosaic symptoms instead of green mosaic on tobacco leaves (23). However, the 126-kDa replicase gene of TMV is the avirulence gene corresponding to the gene *N* controlling a comprehensive hypersensitive response to tobamoviruses in *Nicotiana glutinosa* L. (29). Recently, the gene *N* has been isolated and characterized (43). Finally, mutation of the 30-kDa gene facilitating cell-to-cell spread of ToMV confers an ability to overcome the *Tm-2* gene for extreme resistance in tomato (26). In all of these instances, a change of a few amino acids or even a single nucleotide change in the viral avirulence gene was sufficient to change the host response.

Based on the PVX and TMV models described, we believe that the PVA hypersensitive response in potato also represents a gene-for-gene interaction. Supposing that the CP of PVA may play an important role in the elicitation of the hypersensitive responses in potato cultivars, two regions of the PVA CP are particularly interesting. One is at the N-terminal region, where differences among the three American PVA isolates (PVA-U, PVA-M, and PVA-CAN) and the European isolate PVA-B11 are most prominent. The other region of interest is at the CP amino acids 166, 168, and 169. These areas of CP variability, which seem to be linked to phenotypic differences in host resistance, can be examined directly for their role in the hypersensitive response through development of an infectious cDNA of PVA, and the hypersensitivity genes can be mapped in the respective potato cultivars. These topics will be our primary areas of research emphasis in the future.

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