Detection and Characterization of Peru Tomato Virus Strains Infecting Pepper and Tomato in Peru

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ABSTRACT

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Virus isolates from pepper (*Capsicum baccatum* var. *pendulum*) and tomato (*Lycopersicum esculentum*) in coastal areas of Peru were identified as Peru tomato virus (PTV). Except for four *Chenopodium* spp., which developed local lesions, their host range was confined to the Solanaceae. PTV was not seed transmitted in tomato or pepper. The infectivity of crude sap withstood in vitro aging 10–21 days, had a thermal inactivation point between 55 and 60 C, and a dilution end point between 10^{-5} and 10^{-6}. Highly purified preparations of the most severe strain (isolate M4 from pepper) used as antigen produced antiserum with a titer of 1:16,384. A distant serological relationship was detected between PTV and potato virus Y, tobacco etch, and pepper mottle viruses, but not between PTV and pepper veinal mottle or pepper severe mosaic viruses. Normal length of particles of the four strains of PTV was 741–746 nm. PTV was confirmed as a new potyvirus based on host range and serological properties.

Additional key words: potyvirus, virus serology, PTV characteristics.

In a 1971 survey of viruses infecting tomato and pepper in the coastal area of Peru, isolates were commonly found which induced similar symptoms in a set of indicator plants. These isolates were similar to a virus noted by Raymer et al (16) and Fribourg (8) and called by them Peru tomato virus (PTV). This paper presents evidence on the occurrence of PTV in tomato and, for the first time, in pepper in the coastal areas of Peru. Characteristics of PTV were determined for several isolates and a number of strains were differentiated.

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MATERIALS AND METHODS

Of 15 virus isolates preserved, four were selected for detailed study because they differed in virulence, host range, and geographical origin. These were designated M4, 15, 1-S-9, and 1H. Later, the isolate (H) described by Raymer et al (16) was obtained from H. E. Waterworth, USDA Plant Introduction Station, Glenn Dale, MD 20769. The M4 isolate was from pepper (*Capsicum baccatum* var. *pendulum*), and the others were from tomato. Cultures were maintained in tobacco *Nicotiana tabacum* L. 'Burley 21' plants kept in insect-proof cages in the greenhouse.

Plants were mechanically inoculated by gently rubbing corundum-dusted leaves with a cheesecloth pad dipped in sap extract from infected leaves. Plants were kept without artificial

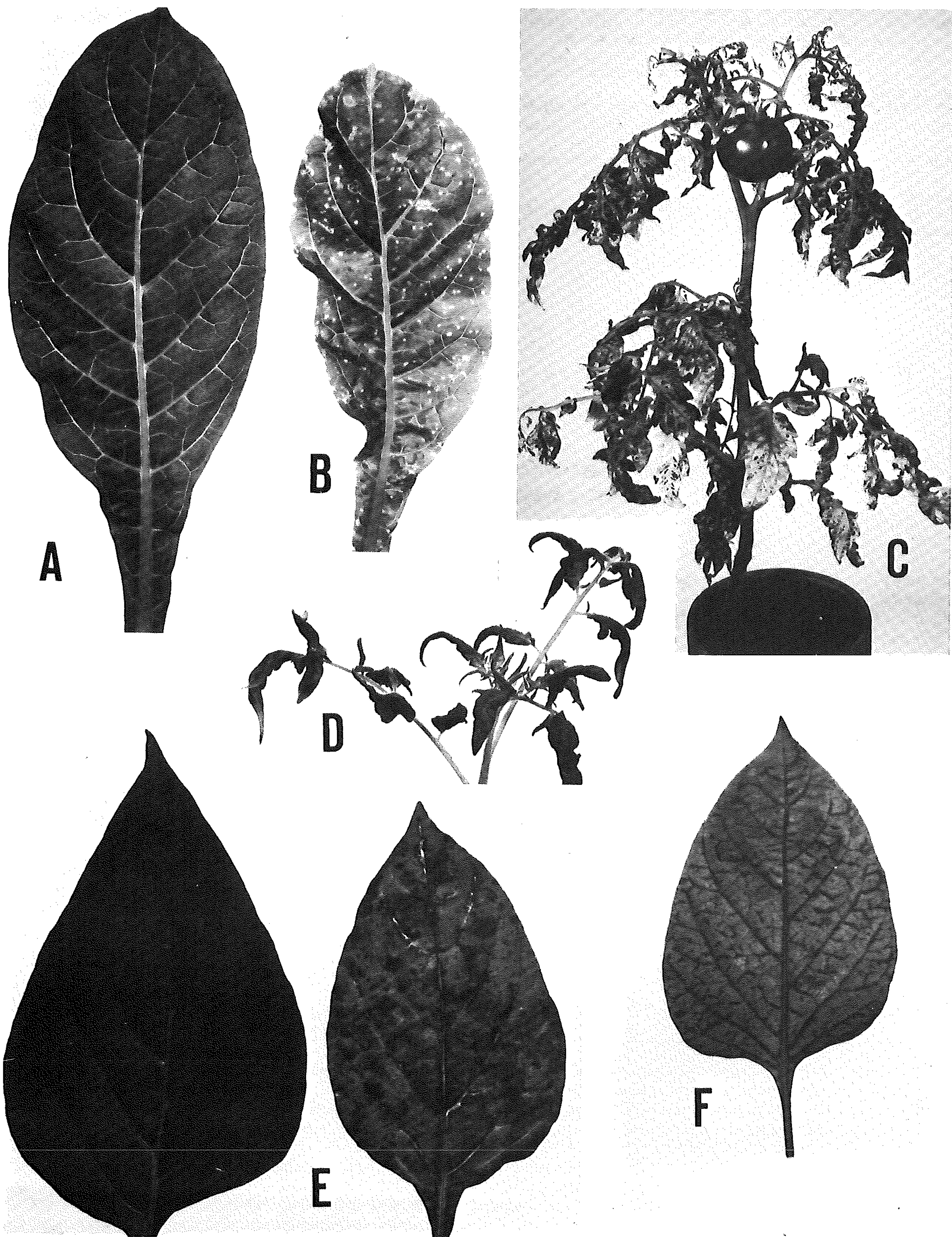

Fig. 1. Symptoms of Peru tomato virus (PTV) in various hosts. **A and B,** Healthy and infected (respectively) *Nicotiana debneyi* showing circular chlorotic spots and some chlorotic rings. **C,** Marglobe tomato showing leaf deformation and extensive necrosis induced by M4, the virulent isolate. **D,** Typical leaf twisting and deformation induced in Marglobe tomato. **E,** Yolo Wonder pepper, healthy and infected with PTV, showing bright yellow veinbanding and mosaic induced by more virulent isolates. **F,** Green veinbanding in Yolo Wonder pepper.

light in an air-conditioned greenhouse at 24

For much of the work, the presence of infective virus in species tested as possible hosts, or in physical property tests, was determined by inoculating Burley 21 tobacco. Comparative tests proved it to be a more sensitive host than *Chenopodium amaranticolor* Coste & Reyn. Symptoms were distinct on Burley 21 tobacco and, since it produced a high concentration of virus, it also was used as a source of virus for purification.

To determine host range, four or more young rapidly growing plants of each species were inoculated with each virus isolate. If no symptoms resulted, back inoculations were made from uninoculated leaves to Burley 21 tobacco. Tests with diagnostic species were repeated several times.

PTV was purified from Burley 21 leaves, harvested 2–4 wk after inoculation of six- to eight-leaf plants. After cooling overnight at 4 C, leaves were homogenized for 2 min in a Waring Blendor containing (proportionally) 1 g of leaf: 1 ml of chloroform: and 2 ml of 0.5 M borate buffer (pH 7.6) containing 0.2% 2-mercaptoethanol. After low-speed centrifugation (LSC) (8,000 rpm in a Sorval GSA rotor, or 10,000 rpm in a Spinco No. 30 rotor), the supernatant liquid was passed through filter paper. Virus was then precipitated by adding 50 g/L polyethylene glycol-6000 and NaCl to make 0.2 M and stirring the mixture for 1 hr. The precipitate was pelleted by LSC and then dispersed by shaking for 2 hr in an amount of 0.05 M borate and 0.01 M EDTA (pH 7.5) equal to about one-fourth of the original volume. After LSC to remove insoluble material, the preparation was given two cycles of differential centrifugation (30,000 rpm in a Spinco No. 30 rotor, or 32,500 rpm in a Spinco No. 40 rotor) (HSC) and LSC. Pellets after the first HSC were resuspended in 0.1 M borate and 0.01 M EDTA (pH 7.8) and in 0.1 M borate (pH 7.8) after the second HSC.

PTV was further purified by rate-zonal centrifugation in 7–25% sucrose linear density gradients in 0.1 M borate, pH 7.8. After 1.5 hr at 21,000 rpm in a Spinco SW 25.1 rotor, an ISCO fractionator was used to detect and recover the virus band. The virus was freed of sucrose by HSC and resuspended in 0.05 M borate, pH 7.8; it is referred to as a "zonal preparation."

Appreciable quantities of PTV pelleted at the bottom of density gradient tubes. This also was resuspended in borate buffer, given HSC and LSC and is referred to as a "pellet preparation."

For electron microscopy, leaf dips were negatively stained with 1% potassium phosphotungstate (PTA) at pH 6.8. Purified virus was negatively stained with 2% PTA or shadowed with carbon-platinum. The magnification of the JEM 7 electron microscope was checked periodically with a carbon replica of a diffraction grating with 2,160 lines per millimeter. Highly purified TMV also was used as an internal size standard.

Antiserum for PTV isolate M4 was prepared by intramuscular injection of a rabbit twice a week for 6 wk with 1 mg of virus in 1 ml, emulsified in 1 ml of Freund's incomplete adjuvant. The rabbit was bled weekly, beginning in the 5th wk.

Microprecipitin and sodium dodecyl sulfate (SDS)-agar double diffusion tests were done in plastic 100 × 15-mm petri dishes. The agar was 0.8% Ionagar No. 2, containing 1% NaN_3 and 0.3% SDS. Five-millimeter-diameter wells were spaced 6 mm apart.

RESULTS

Host range and symptomatology. Our results were similar to those of Fribourg (8) in showing that, except for a few chenopodiaceous species, the host range of PTV was confined to the Solanaceae. Species found susceptible which were not reported by Fribourg (8) are: *Capsicum baccatum* var. *pendulum* (Willd.) Eshbaug; *C. chinense* Jacq.; *C. frutescens* L. *'Tabasco'; Datura meteloides* DC.; *Lycopersicum hirsutum* H.B. & K.; *Nicotiana acuminata* (R. C. Grah.) Hook.; *N. Goodspeedii* Wheeler; *N. gossei* Domin.; *N. knightiana* Goodsp.; *N. longiflora* Cav.; *N. multivalvis* Lindl.; *N. rustica* L.; *N. sylvestris* Speg. & Comes; *N. tabacum* L. (seven cultivars); *Petunia hybrida* Rydb.; *P. inflata* R. E. Fries; *Physalis angulata* L.; *P. peruviana* L.; *P. philadelphica* Lam.; and *Solanum sisymbriifolium* Lam.

Four *Chenopodium* spp. reacted with local lesions: *Chenopodium album* L., *C. quinoa* Willd., and *C. urbicum* L. developed chlorotic local lesions and *C. amaranticolor* reacted with necrotic local lesions.

Uninfected *solanaceous* species included *C. annuum* L. Agronomico 8, Ac 1534, Ac 2120, Ac 2207, P 11, and P 34; *Datura stramonium* L.; *Nicotiana benavidesii* Goodsp.; *S. tuberosum* 'Katahdin'; and *S. demissum* × *S. tuberosum* clone A6.

No infection was obtained in any species or cultivars in the

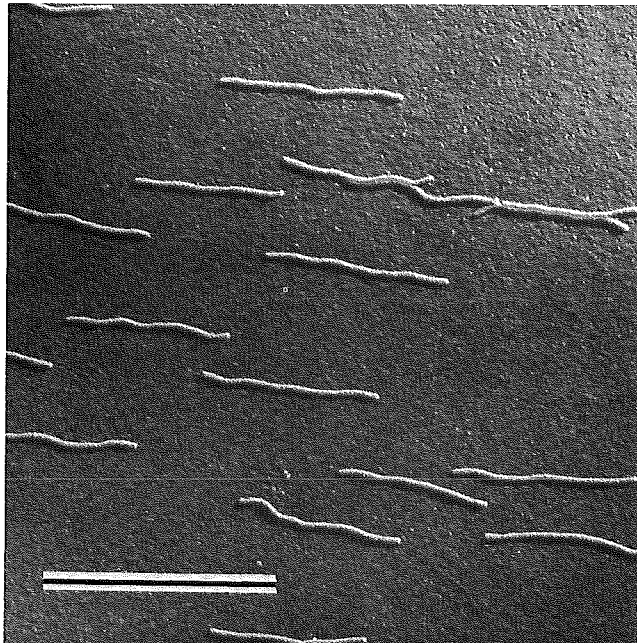

Fig. 2. Electron micrograph of a platinum-carbon-shadowed purified preparation of Peru tomato virus after rate density gradient centrifugation. Bar represents 1,000 nm.

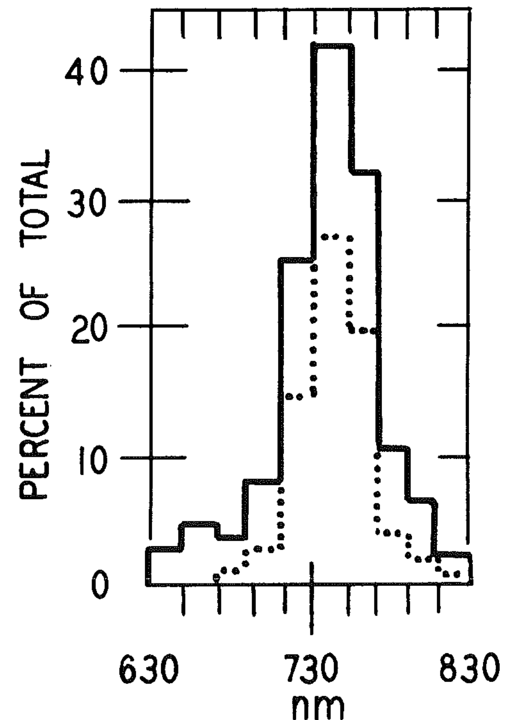

Fig. 3. Particle length distribution of negatively stained purified preparations of Peru tomato virus isolates M4, 1H, 15, and 1-S-9. The dotted line indicates the lowest percentage for any strain, the solid line the highest percentage.

Amaranthaceae, Apocynaceae, Caryophyllaceae, Compositae, Convolvulaceae, Cruciferae, Cucurbitaceae, Eurphorbiaceae, Gramineae, Iridaceae, Labiatae, Leguminosae, Malvaceae, Pedaliaceae, or Umbelliferae.

On many *Nicotiana* spp. veinclearing at the apex of upper leaves was the first symptom. Leaves later developed circular chlorotic spots. These symptoms were characteristic of PTV, particularly in *N. debneyi* (Fig. 1). Color breaking occurred in Burley 21 and colored *Petunia* spp. petals. Symptoms in tomato and pepper, which developed mosaic, then a green veinbanding, are shown in Fig. 1.

The four isolates studied were similar in host range; M4 was the most virulent. The PTV isolates differed from other viruses reported occurring naturally in tomato, pepper, potato, tobacco, and datura (5,7,18), in host range, and in symptoms produced.

Physical properties. The dilution end point of isolate M4 was between 10^{-5} and 10^{-6}; the thermal inactivation point was between 55 and 60 C. These figures are higher than reported by Fribourg (8) and are probably the result of testing by inoculation to the very susceptible Burley 21 tobacco. Longevity in vitro of the 15 isolates range from 10 to 21 days. Burley 21 leaf tissue desiccated over $CaCl_2$ was still infective after 3 yr.

Seed transmission. No seed transmission was detected in 1,314 Marglobe tomato seeds, 281 California Wonder, or 400 Escabeche pepper seeds, although all fruits, mottled or not, contained infective virus.

Virus purification. Virus, purified by density gradient centrifugation, had a UV absorbance spectrum typical of a rod-shaped virus. The absorbance minimum at 244 nm was at a slightly lower wavelength than the 245–247 nm minimum reported for some potyviruses (4,12,13,19). This might indicate a higher proportion of nucleic acid or guanine. The $A_{260/280}$ ratio was 1.28 ± 0.01 for zonal preparations and 1.27 ± 0.01 for pellet prepartions, uncorrected for light scattering. (All standard deviations reported are from 11 different preparations). These ratios are well within the range of 1.20 to 1.37 reported for other potyviruses (12,13,17). The nucleic acid content of PTV was calculated as 7% (11).

Virus yields, based on an extinction coefficient, $E^{0.1\%}_{1\,cm}$ 260 nm, of 2.8 (19) was 14 mg/kg fresh leaves during fall and winter, and 40–50 mg/kg during spring and summer. Virus resuspended from the bottom of density gradient tubes, and which was not removed by LSC, accounted for about 45% of the yield. Both zonal and pellet preparations showed in the electron microscope (Fig. 2) few or no small contaminant particles indicating minimal breakage of virions.

With the same procedure as for PTV isolate M4, three other PTV isolates, tobacco etch, pepper mottle, and three isolates of PTV were readily purified.

Electron microscopy. Virus particles were measured on enlarged prints of negatively stained grids, classified as to length at intervals of 10 nm, and histograms of the particle length distribution were prepared. From the main maximum the arithmetic mean particle length, or normal length (NL) of each isolate was calculated (3). The NL of 1,301 particles of the four PTV isolates varied from 741 to 746 nm (Fig. 3). Particle width was 11–13 nm. An NL of 741–746 nm is consistent with grouping PVT with the potyviruses (10).

Determinations of NL in leaf dip preparations from infected *Capsicum baccatum* var. *pedulum* were essentially the same as those from purified preparations.

Serological relationships. Antiserum to PTV isolate M4, obtained from a bleeding 2 wk after the 12th (last) injection, had a titer of 1:16,384. Eight weeks after the last injection, the antiserum had a titer of 1:2,048 as determined by microprecipitin test. Both antisera were selected for determination of the serological relationships of PTV by SDS-agar double diffusion, but only the highest titer antiserum was used for microprecipitin tests.

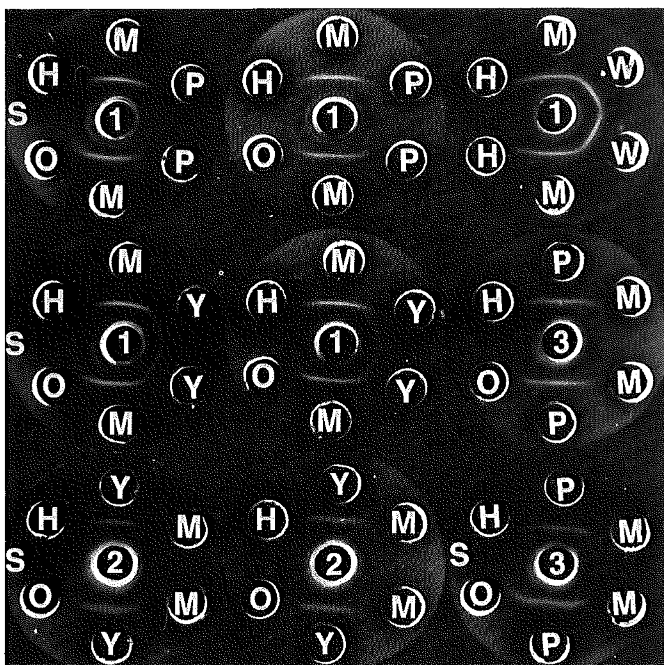

Fig. 4. Immunodiffusion tests in SDS-agar. Serological relationships of Peru tomato virus (PTV), and the effect of excess SDS on nonimmunological precipitations. Patterns not identified with S are extracted with crude sap without SDS; patterns identified with S, extracted with SDS. Center wells contained antiserum (As): (well 1) PTV, M4-9 As; (well 2) potato virus-Y M^sM^r As; and (well 3) pepper mottle virus As. Peripheral wells contained freshly extracted sap of tobacco from plants infected with: PTV M4 isolate (M); PTV-W isolate H (W); potato virus Y-M^sM^r (Y); or pepper mottle virus (P); or from healthy plants (H); or (O) are empty. Note in (1) the nonimmunological precipitin line in front of the empty well (O) produced by the M4-9 As. This line only was present in front of the healthy sap well (H) when sap was SDS-treated (patterns S). All antisera produced a second nonimmunological precipitin ring at the edge of the As well.

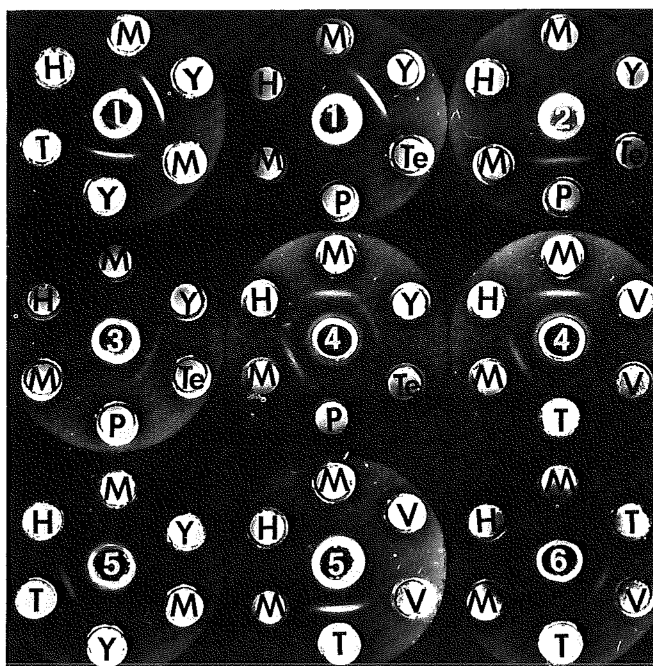

Fig. 5. Immunodiffusion tests in SDS-agar. Serological relationships of Peru tomato virus (PTV). Center wells contained antiserum to: (well 1) potato virus Y-N^sN^r; (well 2) pepper mottle virus; (well 3) tobacco etch virus; (well 4) PTV isolate M4; (well 5) tobacco vein mottling; and (well 6) pepper veinal mottle virus. Peripheral wells contained freshly extracted sap of tobacco infected with PTV M4 isolate (M), potato virus Y (Y), pepper mottle virus (P), tobacco etch virus (Te), tobacco vein mottling virus (T), or from healthy plants (H). Peripheral wells (V) contain freeze-dried material extracted from a pepper veinal mottle virus-infected plant, stored frozen, and resuspended in water.

The four PTV isolates, in SDS-agar double diffusion tests, gave reactions of antigenic identity among themselves and with PTV-W (Fig. 4). Tests were done with PTV antiserum and the viruses listed in Table 1, as well as with bean yellow mosaic virus (BYMV), lettuce mosaic virus (LMV), pepper severe mosaic virus (PSMV), and pepper veinal mottle virus (PVMV). The reciprocal tests also were done with antisera to most of the viruses in Table 1. Weak reactions of partial identity usually were obtained in tests with PVY and antiserum to isolate M4 (Fig. 4). With PeMV and TEV, reactions of partial identity were very weak, indicating a distant serological relationship, and usually appeared only when PTV-M4 antiserum was used (Fig. 5). A reaction of partial identity between BMoV and M4 occurred only with BMoV antiserum. No reactions were obtained with M4 antjserum and BYMV, LMV, PVMV, TVMV, TuMV, or WMV-2. Antigens of BYMV and LMV were not available and thus were not tested with PTV antiserum.

Sap extraction with 3% SDS, as specified by Purcifull et al (15), enhanced the reactions of partial identity (Fig. 4), but resulted in nonimmunological precipitation when M4 antiserum was used. This was evident in front of empty wells left as controls (Fig. 4, patterns S), and is evidently due to excess SDS.

In microprecipitin tests, the titer of each antierum in homologous and heterologous combination, and its behavior against concentrated host nucleoprotein were determined. All antisera (and the saline control) produced a white granular precipitate when mixed with host nucleoprotein at an A_{260} of 1.3 to 20. This was readily differentiated from the flocculent virus-antibody precipitate. The highest homologous or heterologous titer occurred with about the same antigen concentration, 3.9 μg/ml, and this concentration was therefore used in further comparative tests. The extent of cross reactivity was expressed as a serological differentiation index (SDI), defined as the number of twofold dilution steps separating homologous and heterologous titers (20). There were no serological differences (SDI = 0) among the four PTV isolates. There were SDI's of 5–8 between PTV-M4 and PVY-M^sR^s, PVY-N^sN^r, PeMV, and TEV. PeMV and TEV appeared more distantly related to PTV than did the PVY strains.

Eleven other isolates from Peruvian tomato and pepper also were identified as PTV on the basis of serology and differential hosts. PTV is evidently widely distributed in the coastal region of Peru. It has not been reported previously in pepper.

Strains of PTV. Four strains of PTV could be differentiated by severity of reactions of *C. annuum* 'Avelar' and *Lycopersicum esculentum* 'Marglobe.' Strains M (isolate H) and N (isolate M4) infected *C. annuum* 'Avelar' and *C. chinense* PI 159236, while the O strains (isolates 1H, 15, and 1-S-9) did not. These were differentiated by symptoms in tomato. *C. annuum* 'Agronomico 8', P 11 (PI 264281), P 34 (SC 46252), Acc 2120 (PI 342947) and Acc 2207 were not infected by PTV strains. The reactions of lines of *Capsicum* to these strains further differentiates PTV strains from other potyviruses occurring in pepper, since the lines have been reported as differentials for various pepper viruses (21).

DISCUSSION

On the basis of host range, properties, particle morphology and length, and serological relationships of four isolates, the Peru tomato virus reported by Raymer et al (16) from tomato is considered to be a new potyvirus.

The interrelationships of members of the potyvirus group are complex and difficult to determine. The immunity of *Datura stramonium* and aphid transmissibility have been considered characteristic of PVY, but it has become clear that these characteristics are shared with some other members of the group. The large number of apparently distinct viruses in the group emphasizes the need for subdivisions. We suggest that particle length may be useful for this purpose.

Brandes (2) found it possible to distinguish between viruses whose normal lengths differed by 10–20 nm. Edwardson (6) pointed out that this estimate has not been challenged. Data selected from his report show that normal length determinations made for many potyviruses in crude sap agree well with

TABLE 1. Sources of antigens used for comparative serological testing of Peru tomato virus isolates

Antigen	Isolated from:	Geographical origin	Donated by:
Bidens mottle virus (BMoV)[a]		Florida, USA	D. E. Purcifull
Healthy tobacco[a]		Florida, USA	D. E. Purcifull
Pepper mottle virus (PeMV)	*Capsicum annuum*	Florida, USA	D. E. Purcifull
Pepper veinal mottle virus (PVMV)[a]	*C. annuum*	Ghana, Africa	D. E. Purcifull
Peru tomato virus (PTV-W, Isolate H)	Tomato	Trujillo, Peru	H. E. Waterworth
Potato virus Y (PVY)	Tobacco	Wisconsin, USA	R. W. Fulton
	Tomato	Florida, USA	D. E. Purcifull
M^sM^r	Tobacco	North Carolina, USA	G. V. Gooding, Jr.
N^sN^r	Tobacco	North Carolina, USA	
Tobacco etch virus (TEV)	Tobacco	Kentucky, USA	R. W. Fulton
Type strain (ATCC)[a]			D. E. Purcifull
Tobacco vein mottling virus (TVMV)	Tobacco	North Carolina, USA	G. V. Gooding, Jr.
Turnip mosaic virus (TuMV)		Wisconsin, USA	R. W. Fulton
Watermelon mosaic virus-2[a] (WMV-2)			D. E. Purcifull
Healthy pumpkin[a]			D. E. Purcifull

[a] Freeze-dried, noninfective antigen.

determinations made on purified preparations. Differences were less than 20 nm in most instances, even when reported by different authors. The variation for PTV isolates reported here is within 10–20 nm.

Length differences of viruses occurring in tomato and pepper do occur. It seems possible that the potyvirus group might be subdivided on this basis. Three length classes might be defined: PVY, TEV, and PeMV (730–740 nm); PTV, PsMV, and Costa's Brazilian PVY (no serology reported) (740–760nm); and PVMV (770 nm). If this basis for subdividing the group is to have validity, however, precise determination of normal length is necessary.

The SDS-agar double diffusion test has been very useful in detecting and differentiating potyviruses (14). The formation of nonimmunological precipitates, however, emphasizes the need for adequate controls.

The degree of serological relationships among what are considered PVY isolates has not, with some exceptions (1,9) been sufficiently investigated. In our work the microprecipitin tests with purified virus (1) and the SDS-agar immunodiffusion with crude sap (9) correlated well. The antigenic identity shown by four PTV strains in SDS-agar double diffusion corresponded with an SDI of 0 in microprecipitin tests. The weak spur formation indicating partial identity corresponded to an SDI $\geqslant 5$ in microprecipitin tests. This seems a sufficient difference to consider PTV to differ from PVY and other potyviruses occurring naturally in tomato or pepper, particularly since a high-titer antiserum was used.

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