Etiology

Properties and Classification of a Potexvirus Isolated from Three Plant Species in Argentina

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ABSTRACT

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A potexvirus with particles 538 nm long was isolated from *Plantago major, Taraxacum officinale*, and *Callistephus chinensis* in Argentina. The virus, provisionally named Argentine plantago virus (APlaV), has a wide host range and produces systemic symptoms in several species of the Chenopodiaceae, Compositae, Labiatae, Scrophulariaceae, and Solanaceae. The thermal inactivation point is between 90 and 95 C and the infectivity dilution end point is between 10^{-8} and 10^{-9}. Longevity in vitro is between 32 and 64 days. The cytoplasm of infected cells contains banded inclusions consisting of virus particles arranged in parallel and hypertrophied endoplasmic reticulum. Crystal-like inclusions of unknown composition occur in the cytoplasm and in the nuclei. The coat protein of APlaV has a molecular weight of 21.6×10^{-3} daltons. APlaV is serologically related to papaya mosaic, plantago severe mottle, and Boussingaultia mosaic viruses. The average serological differentiation indices in reciprocal tests range from 1.75 to 5.5.

During a survey of viruses affecting weed and ornamental plants in the Mendoza province of Argentina, a virus was isolated in 1980 from *Plantago major* L. in the area of Tunuyán. The plants showed a mild mosaic. In 1981, this virus was found at the same location in *Taraxacum officinale* Weber and in Lujan de Cuyo also in *Callistephus chinensis* (L.) Nees. The latter two hosts showed a more severe mosaic and leaf malformations. The virus proved to be a potexvirus which provisionally was named Argentine plantago virus (APlaV).

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MATERIALS AND METHODS

Host range and transmission studies were done in an insect-proof greenhouse. Virus isolates were maintained in *Nicotiana tabacum* L. 'Xanthi nc' and *Nicotiana debneyi* Domin. Recovery tests were made on *N. debneyi* to detect latent infections in hosts that showed only local reactions or no symptoms after mechanical inoculation.

Myzus persicae Sulz. was tested as a vector of the virus on *Datura stramonium* L. and *N. debneyi* by using 10 aphids per plant. Seed transmissibility was checked with seeds from infected *N. debneyi, N. clevelandii* Gray, and *Datura stramonium* which were sown in sterilized soil shortly after harvest. Seedlings were observed for symptoms and tested 1 mo after emergence by sap inoculation on *Chenopodium quinoa* Willd.. Soil transmission was attempted with soil from the place where infected *Plantago* and *Taraxacum* plants were collected. Seedlings of *C. quinoa* and *Cucumis sativus* L. were used as bait plants. Infection was assessed by symptoms and sap inoculation on *C. quinoa*.

Leaf dip preparations of infected plants of *N. tabacum* 'Xanthi' or 'Samsun' were stained with 2% potassium phosphotungstate, pH 7.0. Particles were observed with a Zeiss EM 10 C electron microscope at a magnification calibrated with a carbon-grating replica, using the Morphomat 10 image analyzing system. For ultrathin sections, small samples of infected leaves were fixed 15 days after inoculation in 5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) or 0.1 M phosphate buffer, pH 7.2. After postfixation in 1% OsO_4 in the same buffer, the blocks were treated with 2% uranyl acetate in water for 2 hr, dehydrated in acetone, and embedded either in an Epon-Araldite mixture or in Epon alone. Sections were cut with a diamond knife, mounted on carbon-coated grids, double stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop or a Zeiss EM 10 C electron microscope.

The plantago isolate of APlaV and Plantago severe mottle virus (PlaSMV) (14), were purified from systemically infected *N. tabacum* L. 'Samsun' and *Lycopersicon esculentum* Mill., respectively, by using a slightly modified silver nitrate-chloroform-polyethylene glycol method (12). The final concentration of silver nitrate in the crude sap was increased from 0.033 to 0.052%. The protein molecular weights were determined by electrophoresis in 2.5 and 7.5% polyacrylamide gels containing 0.1% SDS (13). Rabbits were immunized with purified virus by two intramuscular injections in Freund's complete and incomplete adjuvant spaced 1 wk apart. Bleedings were taken at 2-wk intervals. Titer determinations were done by the drop precipitin test (2).

RESULTS

Host range, stability in sap, and transmissibility. The virus was readily transmitted by sap inoculation and had a wide host range. It infected 35 of 44 species in 10 families (Table 1). No symptoms were observed and recovery tests were negative with *Brassica oleracea* L. 'Botrytis,' *B. perviridis* Bailey, *Carica papaya* L., *Glycine max* (L.) Merr., *Nicotiana glutinosa* L., *Physalis peruviana* L., *Phaseolus vulgaris* L., *Pisum sativum* L. 'Dark Skin Perfection,' and *Sonchus oleraceus*.

In *N. tabacum* 'Xanthi' the virus had a thermal inactivation point between 90 and 95 C, a dilution end point of 10^{-8}, and retained infectivity for 32 days but not 64 days at room temperature.

The virus was not transmitted by *Myzus persicae* to 30 plants of *D. stramonium* and 30 plants of *N. debneyi*. It was not detected in any of 100 plants of *N. debneyi*, 50 plants of *N. clevelandii*, or 80 plants of *E. stramonium* grown from infected seed. No soil transmission was observed with 100 bait seedlings of *Chenopodium quinoa* and *Cucumis sativus*.

Electron microscopy. Dip preparations contained flexuous filamentous particles 538 nm long (normal length of a population of 102 particles). Ultrathin sections of infected mesophyll cells of *N. tabacum* 'Xanthi,' 'Samsun,' or 'White Burley' showed a voluminous cytoplasm with conspicuous inclusions consisting of proliferated rough or smooth endoplasmic reticulum and numerous roundish bodies (Fig. 1A) up to 1 μm in diameter with a crystal-like fine structure (Fig. 1B). Crystal-like inclusions were also observed in the nuclei of infected cells. Virus particles formed clusters or banded arrays (Fig. 2A). In the latter, virus particles were regularly arranged with their ends in one plane (Fig. 2B). Crystal-like inclusions and virus aggregates were not delimited by membranes.

Purification, protein molecular weight, and serological properties. Purified preparations of APlaV were readily obtained and yielded a single protein band in SDS polyacrylamide gel electrophoresis. From the migration rate in 7.5% polyacrylamide gels, a protein molecular weight of $21.6 \pm 0.2 \times 10^3$ daltons (average of seven determinations) was calculated. For the proteins of PLaSMV, Boussingaultia mosaic virus (BoMV) (1) and papaya mosaic virus (PapMV) molecular weights of 22.8×10^3 daltons, 23.2×10^3 daltons and 22.9×10^3 daltons were determined. Almost identical values were obtained with 2.5% polyacrylamide gels indicating that the proteins of these viruses behave normally in SDS polyacrylamide electrophoresis.

APlaV was strongly immunogenic and antisera with titers between 1:1,000 and 1:4,000 in the slide precipitin test were readily obtained. In this test, APlaV did not react with antisera to the following potexviruses: cactus X, clover yellow mosaic, cymbidium mosaic, narcissus mosaic, pepino X, plantain X, potato X, and white clover mosaic. The antiserum to plantain virus X was kindly provided by R. Hull. Reactions were, however, observed with antisera to BoMV, PapMV, and PlaSMV and sera to APlaV

TABLE 1. Host range and symptomatology of Argentine plantago virus

Family, genus, and species	Symptoms[a] Local	Systemic
Amaranthaceae		
Gomphrena globosa L.	NS	-
Brassicaceae		
Brassica rapa L. 'Blanco chato'	-	(sl)
Matthiola incana (L.) R. Br. 'Gigante de Niza'	-	(sl)
Chenopodiaceae		
Beta vulgaris L.	NS	CR, CL, M
Chenopodium amaranticolor Coste et Reyn.	NS	VC, M, N
C. quinoa Willd.	NS	LD, N, M
Spinacea oleracea L.	-	M
Compositae		
Calendula officinalis L.	-	(sl)
Callistephus chinensis (L.) Nees.	-	M
Lactuca sativa L. 'Great Lakes'	NS	(sl)
Taraxacum officinale Weber		M, LD
Zinnia elegans Jacq.	-	M
Cucurbitaceae		
Cucumis sativus L.	NS	(sl)
Labiatae		
Ocimum basilicum L.	NS, NR	CR, NR, NL
Leguminosae		
Vicia faba L.	-	(sl)
Vigna sinensis (Torner) Savi 'Blackeye'	CS	(sl)
Plantaginaceae		
Plantago lanceolata L.	-	(sl)
Plantago major L.		M
Scrophulariaceae		
Antirrhinum majus L.	-	M
Solanaceae		
Capsicum annuum L. 'Calahorra'	CS, NS	(sl)
Datura metel L.	CS, NS	VC, M, N
Datura stramonium L.	NS, NR	NL, M, N
Lycopersicon esculentum Mill. 'Rossol'	-	M, N
L. pimpinellifolium Mill.	-	M
Nicandra physaloides (L.) Pers.	NS	M
Nicotiana clevelandii Gray	NS, NR	NR, NL, LD
N. debneyi Domin.	NS	VB, M, LD
N. rustica L.	NS, NR	NR, NL, M
N. sylvestris Speg. et Comes	NS, NR	NR, NL, M
N. tabacum L. 'Samsun,' 'Turkish,' and 'Xanthi n.c.'	NS	NR, NL, M
N. tabacum L. 'White Burley'	CS, NS	NR, NL, M
Petunia hybrida Vilm.	CS	M, LD, N
Physalis floridana Rydb.	-	M, LD

[a] Abbreviations for symptoms: CL = chlorotic line pattern; CR = chlorotic rings; CS = chlorotic spots; LD = leaf deformation; M = mosaic; N = necrosis; NL = necrotic line pattern; NR = necrotic rings; NS = necrotic spots; VB = vein banding; VC = vein clearing; and (sl) = symptomless infection.

reacted with these viruses. The approximate average serological differentiation indices of reciprocal tests (RT-SDIs) (10,15) were determined with a limited number of bleedings taken from rabbits immunized with these viruses over periods from 16 to 39 wk (Fig. 3). SDIs tended to become smaller during the course of immunization (Table 2).

DISCUSSION

Plantago species are known to be susceptible to many viruses (9). In Argentina, until the present, only broad bean wilt virus was reported in *Plantago lanceolata* L. (7); in *Callistephus chinensis* cucumber mosaic virus was found (6) and *Taraxacum officinale* has so far not been identified as a naturally infected virus host in Argentina.

The virus isolated by us has typical properties of a potexvirus (11), ie, filamentous particles 538 nm long, lack of aphid and soil transmissibility, high concentration in sap from infected plants, formation of banded inclusions in the cytoplasm of infected cells and a protein molecular weight of 21.6×10^3 daltons. The thermal inactivation point (90–95 C) and the dilution end point (10^{-8}) are unusually high for a potexvirus. The virus is serologically related to the established potexviruses PapMV, PlaSMV, and BoMV, but not to plantain virus X (9) and others.

There are many similarities in the host ranges of APlaV and BoMV, the virus to which APlaV is most closely related

TABLE 2. Homologous and heterologous antiserum titers and serological differentiation indices (SDIs) of antisera to Argentine plantago virus (APlaV) obtained from a rabbit after different periods of immunization

Time (wk) after first injection	APlaV antiserum titers[a] with				SDI[b] for APlaV and		
	APlaV[c]	BoMV	PapMV	PlaSMV	BoMV	PapMV	PlaSMV
6	2,048	256/512	128	32	2–3	4	6
8	2,048	256	128	32	3	4	6
10	2,048	256/512	256	32	2–3	3	6
14	2,048	512	256	128	2	3	4
22	2,048	512	256/512	256/512	2	2–3	2–3

[a] Reciprocal values.
[b] The SDI denotes the number of twofold dilution steps separating heterologous and homologous titers of an antiserum (9,14).
[c] Purified preparations of APlaV, Boussingaultia mosaic virus (BoMV), papaya mosaic virus (PapMV), and plantago severe mottle (PlaSMV) virus adjusted to a concentration eight times as high as their precipitin titers.

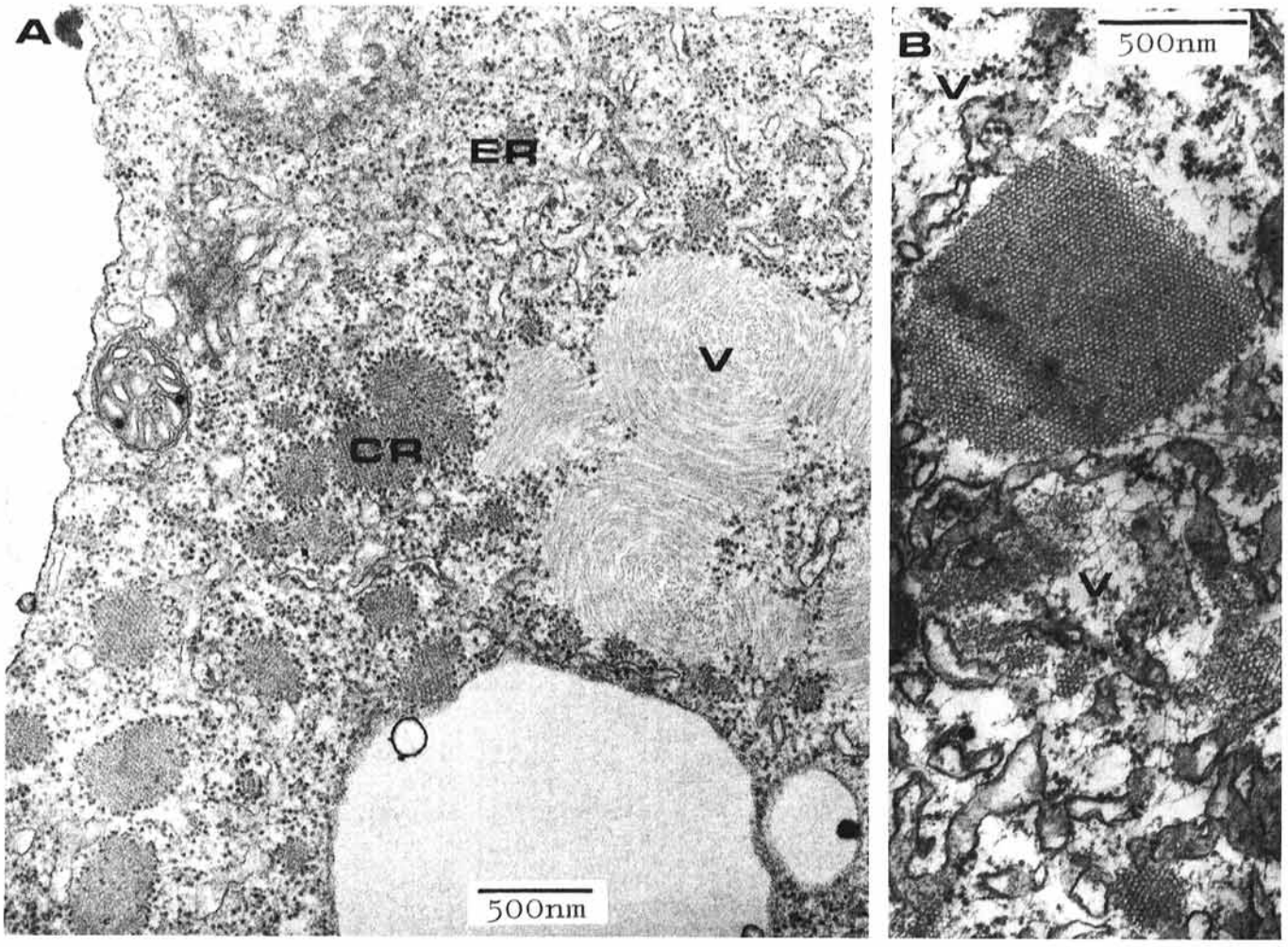

Fig. 1. Ultrathin sections of mesophyll cells of Argentine plantago virus-infected plants of *Nicotiana tabacum* 'Samsun' showing cytological alterations of the cytoplasm. **A**, Hypertrophied endoplasmic reticulum (ER), crystal-like inclusions (CR), and virus particle aggregates (V). **B,** Detail of crystal-like inclusions of various sizes. Note scattered filamentous virus particles (V) between ER elements and inclusions.

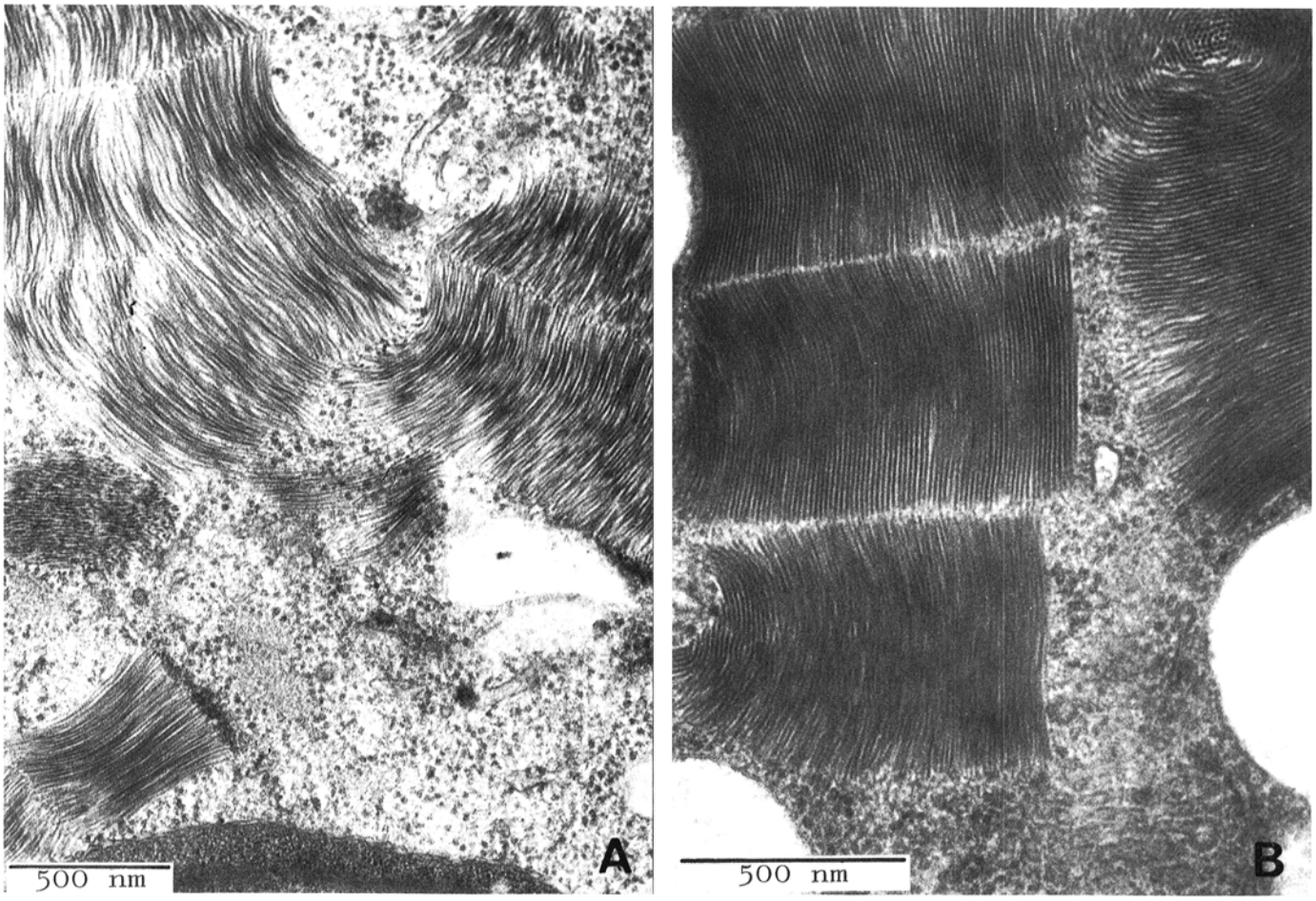

Fig. 2. Ultrathin sections of mesophyll cells of Argentine plantago virus-infected *Nicotiana tabacum* 'Xanthi,' showing banded inclusion bodies in the cytoplasm. **A,** Bands of particles in disordered stacks and **B,** side-by-side aggregate with particle ends in one plane and whirl-like areas.

serologically (Fig. 3). They have 16 susceptible species in common and both produce a systemic mosaic in *Chenopodium quinoa* and *C. amaranticolor*. These hosts are not infected systemically by the type strain of PapMV and PlaSMV; a recently described Ullucus strain of PapMV (3) produces symptomless systemic infections. APlaV in addition gives systemic symptoms in many solanaceous hosts, ie, *Datura stramonium*, cultivars of *Nicotiana tabacum*, *Nicotiana rustica*, and *Petunia hybrida* which are not or only locally invaded by PlaSMV, BoMV, and the type and Ullucus strains of PapMV. PlaSMV and the type strain of PapMV (5) have narrower host ranges than APlaV, but APlaV does not infect papaya.

APlaV induces crystal-like inclusions in the cytoplasm and the nuclei of infected cells; these have not been reported for other potexviruses. However, nuclear inclusions resembling those which have been described for PapMV (4) have not been detected with APlaV.

A study group of the International Committee on Taxonomy of Viruses (8) recently suggested that viruses with different host ranges and an RT-SDI of more than 3 should be regarded as separate viruses. In Fig. 3, the RT-SDI for the pair APlaV–PlaSMV is about 4, indicating that these two isolates which have rather different host ranges might be regarded as separate viruses. The pair APlaV–PapMV has an approximate RT-SDI of 3, which makes it more difficult to decide whether the two viruses should be regarded as strains of one virus or as two separate viruses. This decision becomes especially difficult, since BoMV apparently forms a serological link between APlaV and PapMV (Fig. 3). BoMV has recently been considered to be a strain of PapMV (3), a conclusion which is supported by the low RT-SDI found in our experiments. In a preliminary experiment, an antiserum to the Ullucus strain of

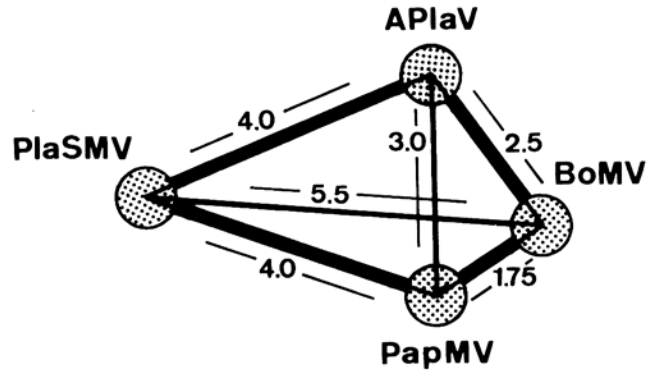

Fig. 3. Serological interrelationships between Argentine plantago virus (APlaV), plantago severe mottle (PlaSMV), Boussingaultia mosaic (BoMV), and papaya mosaic viruses (PapMV). Serological differentiation indices of reciprocal tests are depicted as length units. The system is based on 100 titer determinations with 12 bleedings from two rabbits immunized with APlaV, seven bleedings from two rabbits immunized with PlaSMV, four bleedings from one rabbit immunized with PapMV, and two bleedings from two rabbits immunized with BoMV. The sera to BoMV were kindly provided by L. Beczner.

PapMV (3) kindly provided by A. A. Brunt reacted about equally well with APlaV, BoMV, PapMV, and PlaSMV. These observations indicate that all of these virus isolates that cause different diseases in different hosts form a cluster of serologically more or less closely related viruses for which a clear-cut distinction between 'strains' and 'viruses' is not possible. Such clusters have

long been known in several groups of plant viruses including the tymo- (10), tombus-, tobamo-, and potyvirus groups. We conclude that clusters can occur also in the potexvirus group in which most of the serological relationships reported previously (11) were rather distant.

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