

Potato Virus X — a New Report from Turnip (*Brassica rapa* L.) in India

ABDUL SAMAD*, Q. A. NAQVI, I. D. GARG and J.-C. THOUVENEL

Author's addresses: ABDUL SAMAD and QAMAR A. NAQVI, Plant Virology Laboratory, Department of Botany, Aligarh Muslim University, Aligarh (India). I. D. GARG, Central Potato Research Institute, Simla (India). J.-C. THOUVENEL, Laboratoire de Phyto-virologie, Institut International de Recherche Scientifique pour le Développement à Adiopodoumé, B.P. V-51, Abidjan, Ivory Coast.

With 4 figures

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Abstract

A virus isolated from turnip in Aligarh, India, which caused mild mosaic, mottling and curling of leaves followed by overall stunting of plants, was characterized as potato virus X (PVX) on the basis of its host range, biological and physical properties, particle morphology, ultrastructural studies, and serological relationship.

Zusammenfassung

Potato virus X — ein neuer Befund bei der Stoppelrübe (*Brassica rapa* L.) in Indien

Isoliert wurde ein Virus aus Stoppelrüben in Aligarh, Indien, das schwache Mosaikbildung, Fleckenbildung und Blattrollen und außerdem eine allgemeine Verkleinerung der Pflanzen verursacht. An Hand dessen Wirtsspektrum, biologischer und physikalischer Merkmale, Partikelmorphologie, ultrastrukturellen Untersuchungen sowie serologischen Verwandtschaften wurde das Virus als das potato virus X (PVX) charakterisiert.

Many viruses have been isolated from turnip (*Brassica rapa* L.). These include: turnip mosaic virus (TOMLINSON 1970), turnip yellow mosaic virus (MATTHEWS 1980), turnip rosette virus (HOLLINGS and STONE 1973), turnip crinkle virus (HOLLINGS and STONE 1972), cauliflower mosaic virus (SHEPHERD 1981) and beet western yellows virus (TIMMERMAN *et al.* 1986).

* Present address of ABDUL SAMAD: Phytopathology lab., Institut International de Recherche Scientifique pour le Développement à Adiopodoumé, B.P. V51, Abidjan, COTE D'IVOIRE.



A survey during 1983—1984 in and around the Aligarh district of India, revealed a disease of turnip to be widespread. Affected plants showed a mild mosaic with mottling, deformation and distortion of the leaves; these symptoms did not correspond with any previously reported virus disease of turnip. An overall stunting of the plants and some abnormalities in flowering were also noted, while the roots were poorly developed. Symptoms were very conspicuous in the mature crop and disease incidence ranged between 40 to 80 %. We found that the causal agent of this disease was a potexvirus, serologically related to potato virus X (PVX) and may be a hitherto unreported strain of PVX. PVX has not previously been noted as important in turnip crop (VARMA 1988). Isolation, transmission, identification and some properties of the virus are reported here.

Materials and Methods

Source and maintenance of virus inoculum

Leaves from naturally infected turnip plants were ground up in a sterilized mortar and pestle with 0.1 M phosphate buffer, pH 7.0, containing 0.1 % sodium sulphite. The homogenate was used to inoculate carborundum-dusted leaves of *Nicotiana glutinosa*, *Chenopodium amaranticolor* and other *Nicotiana* spp. The virus isolate was maintained by sap inoculation on a group of healthy and uniform plants of *N. glutinosa* at the 4—5 leaf stage. Periodic checks were made on the assay host, *C. amaranticolor*, to ensure the biological purity of the virus.

Disease incidence

Between October 1983 and March 1984 the level of disease incidence was obtained by monitoring the percentage of plants showing symptoms.

Host range and transmission studies

Several species of plant from different families were assessed for their susceptibility to the virus by mechanical inoculation in a greenhouse with partially controlled temperature and lighting. At least three plants of each species/cultivar were inoculated and a similar number of plants were used as controls. Plants were observed until six weeks after inoculation and the development, sequence and severity of symptoms were recorded. Material from symptomless plants was back-inoculated to *C. amaranticolor* to test for latent infection.

Various possible methods of transmission were tested, such as by dodder (*Cuscuta reflexa* L.), through soil, by aphids and via seeds.

For the aphid transmission studies, colonies of virus-free aphids were raised on suitable plants. Four species of aphid *Aphis gossypii*, *A. fabae*, *Brevicoryne brassicae*, and *Myzus persicae* were used in the tests (for both persistent and non-persistent transmission) and were reared on *Cucumis sativus*, *Solanum nigrum*, *Brassica oleracea* var. capitata and *N. tabacum* cv. White Burley respectively.

Seed transmission tests were done with infected *B. rapa*, *N. glutinosa*, *N. sylvestris*, *Datura metel*, *D. stramonium* and *Trichosanthes anguina* plants in a greenhouse.

Biological Properties

Crude sap obtained from infected *N. glutinosa* leaves was used to determine the dilution and point (DEP), thermal inactivation point (TIP) and longevity *in vitro* (LIV) following the procedures detailed by NOORDAM (1973).

Purification

The virus was purified from systemically infected *N. glutinosa* leaves harvested from plants inoculated 2 weeks previously. Purification involved extraction of sap from infected *N. glutinosa* leaves in 0.1 M phosphate buffer, pH 7.5, containing 0.1 % sodium sulphite and 0.01 M EDTA,

clarification with a mixture of 20 % chilled butanol and chloroform (1 : 1), centrifugation at 6,000 rpm for 10 min and stirring of the aqueous phase with 1 % Triton-X100 for 30 min, followed by two cycles of differential centrifugation with resuspension of the virus pellet. Relative infectivity of the virus at each step of purification was determined by assaying on *C. amaranticolor*. Further purification of the partially purified virus obtained by the above procedure was attempted by centrifugation in a 10—40 % sucrose gradient (BRAKKE 1960) at 25,000 rpm for 3 h.

The virus zone was concentrated by ultracentrifugation and the re-suspended pellet was scanned (220 nm—300 nm) in a SPB-100 Pye Unicam spectrophotometer and assayed on *C. amaranticolor* to determine infectivity. The characteristic symptoms of the disease were easily reproduced on turnip, when inoculated with the purified virus preparation.

Buoyant density

Appropriate amounts of partially purified virus were mixed with caesium chloride and centrifuged at 40,000 rpm for 12 h using a SW50 rotor. The opalescent band (virus zone) was collected and its refractive index measured with an Abbe-type refractometer at room temperature; the values were then converted into buoyant density using the equation given by SZYBALSKI (1968).

Analytical centrifugation

Sedimentation coefficient studies of the virus were carried out by analytical ultracentrifugation using a Beckman Model E equipped with Schlieren optics and An-D rotor with a single sector cell.

Polyacrylamide gel electrophoresis of virus coat protein

The molecular weight of the viral coat protein was estimated in 10 % (w/v) polyacrylamide gel by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) following the methods described by MAIZEL (1971). The virus was dissociated by heating for 2 min in a boiling water bath in the presence of 0.06 M Tris-HCl buffer pH 6.7, containing 0.5 % (w/v) SDS and 1 % (v/v) 2-mercaptoethanol. Standard proteins of various molecular weights were run simultaneously with the samples.

Electron microscopy

The purified preparation were stained with 2 % uranyl acetate, pH 4.2, and examined in a Philips E 420 electron microscope.

Ultrastructural studies

Pieces of infected *N. glutinosa* leaf were fixed in 3 % glutaraldehyde in 0.1 M phosphate buffer pH 7.2, then post-fixed in 1 % osmium tetroxide in the same buffer. Samples were embedded in epoxy resin (SPURR 1969), and sectioned using an LKB ultratome Nova. Sections were stained with 2 % uranyl acetate and lead citrate (REYNOLDS 1963) and examined in a JEOL JEM 100S electron microscope.

Serology

Purified virus preparations were used to immunize rabbits. One intravenous injection in the ear and two intramuscular injections into the thigh were given 10 days apart to a single rabbit. For the intramuscular injections, the virus preparation was emulsified with an equal amount of Freund's incomplete adjuvant. Blood samples were taken after two weeks.

Double diffusion tests were done using 1 % agarose containing 1 % SDS and 0.85 % NaCl in 0.01 M Tris-HCl buffer, pH 8.0. Chloroplast agglutination (VAN SLOGTEREN 1955) and tube precipitin tests were also performed to determine the titer of the antiserum.

Antisera of potato aucuba (1/1,024), potato X (1/1,024), white clover mosaic (1/1,024), narcissus mosaic (1/512) and papaya mosaic (1/1,024) viruses were obtained from Dr. D. Z. MAAT (Wageningen, The Netherlands). Antiserum of Cymbidium mosaic virus (1/1,024) was obtained from Dr. GENEVIÈVE LEBEURIER (Strasbourg, France), and antisera of potato Y (1/1,024) and potato S (1/1,024) viruses were from Dr. S. M. P. KHURANA (CPRI, India).

Results

Disease incidence and host range

Disease incidence between the seedling stage and the flowering stage during the winter of 1983—1984 ranged from 40—80 % (Fig. 1).

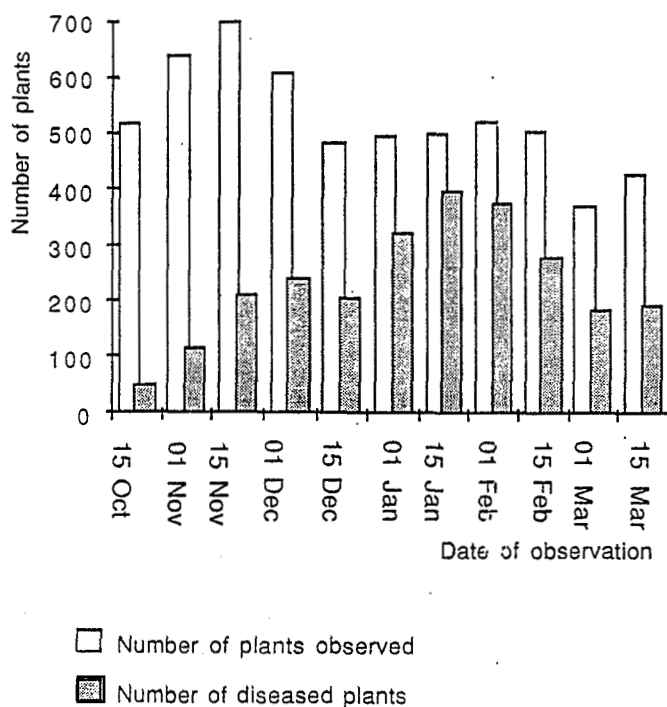


Fig. 1. Disease incidence of the Aligarh-strain of PVX on turnip, during the winter of 1983—1984

The virus from naturally infected turnip was readily sap transmissible to a wide range of hosts and from *N. glutinosa* used as a source plant for host range studies. Of ninety seven plants species belonging to 26 families, sixty species from 19 families were found susceptible, while forty two species belonging to 7 families did not become infected. Most of the susceptible species were members of the Solanaceae, Cruciferae or Asteraceae. No monocotyledons were found susceptible. In most of the *Nicotiana* spp. and cultivars, viz., *N. megalosiphon* and *N. tabacum* cvs. Samsun, Xanthi, White Burley, Bhopali Pakra, CTRI-Special, DR-1, Anand 3, and Harrison's Special, infection took the form of local necrotic lesions encircled by a ring. *C. amaranticolor*, *C. album*, *C. murale*, *C. quinoa*, *Phaseolus lunatus* and *Gomphrena globosa* were also found to produce such local lesions, while the first was found the most suitable host for the quantitative assays. *D. stramonium* and *G. globosa* were recorded as differential hosts.

Similarities between our host range and those previously reported for PVX are shown in Table 1, while differences are given in Table 2. Five solanaceous plants recorded as hosts of PVX were found not to be susceptible to our strain: *Capsicum annuum*, *C. pendulum*, *Lycopersicon esculentum*, *Nicotiana rustica* and *Solanum melongena*.

Transmission

Four species of aphids were tested for their ability to transmit the virus but all results were negative. Neither could the virus be transmitted by dodder, nor via seeds collected from infected plants, nor through soil.

Table 1

Similarities between the host-range of the common strain of PVX and the Aligarh-strain. Abbreviations of symptoms: BP = black patches, BLL = black local lesions, CL = curling of leaves, CLL = chlorotic local lesions, IVY = interveinal yellowing, LAR = leaf area reduction, LD = leaf deformation, LDGP = light and dark green patches, LM = light mosaic, M = mosaic, MF = malformation, MN = marginal necrosis, Mot = mottling, NLER = necrotic lesion encircled by ring, NLL = necrotic local lesion, NL RH = necrotic lesions with red haloes, NS = necrotic spot, RLL = red local lesion, SL = shedding of leaves, St = stunting, VB = vein banding, VC = vein clearing, Y = yellowing, YP = yellow patches

Family, genus and species	Local symptoms	Systemic sympt.
Aizoaceae		
<i>Tetragonia expansa</i> Murr.	—	LM, Y, SL
Amaranthaceae		
<i>Gomphrena globosa</i> L.	NLRH	
Chenopodiaceae		
<i>Chenopodium album</i> L.	CLL	—
<i>C. amaranticolor</i> Coste & Reyn.	CLL	—
<i>C. murale</i> L.	CLL	—
<i>C. quinoa</i> Willd.	CLL	—
<i>Spinacea oleracea</i> L.	—	M, LD
Cucurbitaceae		
<i>Cucumis sativus</i> L.	—	M
Leguminosae		
<i>Vigna sinensis</i> Savi	—	VC, Y, LD
Solanaceae		
<i>Datura metel</i> L.	—	VC, M, Y
<i>D. stramonium</i> L.	—	VC, LDGP, M
<i>Nicotiana benthamiana</i> Domin.	—	M, St, Mot
<i>N. glutinosa</i> L.	—	VC, M
<i>N. sylvestris</i> Speg. & Comes	NLL	VC, LDGP, LD
<i>N. tabacum</i> cvs Samsun, White Burley, Xanthi-nc	NLER	—
<i>Petunia hybrida</i> Vilm.	—	M, Mot, LAR, LD
<i>Physalis floridana</i> Rydb.	—	M
<i>S. nigrum</i> L.	—	LDGP, VB, LAR
<i>S. tuberosum</i> L.	—	M, VC
Umbelliferae		
<i>Apium graveolens</i> L.	—	M, St, CL

Table 2
New hosts and symptomatology observed for the Aligarh strain of PVX. Same abbreviations as in Table 1

Family	Genus and species	Local symptoms	Systemic sympt.
Acanthaceae	<i>Peristrophe bicalyculata</i> Nees	—	LDGP, LAR
	<i>Ruellia tuberosa</i> Kurz.	—	VB, IVY
Aizoaceae	<i>Trianthema portulacastrum</i> L.	NLL	—
Amaranthaceae	<i>Amaranthus tricolor</i> L.	—	LD, M, BP, MN
Asteraceae	<i>Ageratum mexicanum</i> Sims.	—	M, CL, St
	<i>Arctotis stoechadifolia</i> Berg. cv. Sutton Special Hybrid	—	VC, LDGP, CL
	<i>Brachycome iberidifolia</i> Benth.	—	MF, St, CI
	<i>Calendula officinalis</i> L. cvs Orange Conrot, Art Shades	—	LDGP, M, CL, DL, Mot
	<i>Carthamus Roseus</i> L. cv. Dwarf Rose Pink	—	CL, M, LD
	<i>Centaurea imperialis</i> Hort.	—	CL, M, LD
	<i>Dimorphotheca aurantiaca</i> DC.	—	M
	<i>Lageria aurita</i> Sch. Bip.	—	M, St
	<i>Tagetes erecta</i> L.	—	CL, LD
<i>Vernonia cinerea</i> Less.	—	CL, LAR, LD	
Balsaminaceae	<i>Impatiens balsamina</i> L.	—	LDGP, M, LAR
Convolvulaceae	<i>Convolvulus major</i> Gimlib cv. Picta	—	M, LAR
Crassulaceae	<i>Bryophyllum calycinum</i> Salisb.	—	M
Cruciferae	<i>Alyssum maritimum</i> Lam.	—	VC, Mot, SL
	<i>Brassica campestris</i> L.	—	CL, LAR, St
	<i>Brassica oleracea</i> L. var. <i>botrytis</i>	—	LDGP, Y
	cv. Snow Ble 16; cv. Katki Faizabadi Kalmi	—	Mot, BP
	cv. Katki	—	LDGP, Y
	cv. Dania; cv. Patna Mid Season	—	LDGP
<i>Brassica oleracea</i> L. var. <i>capitata</i>	—		
cv. Pride of India; cv. Sutton Express; cv. Long Blood Red	—		

	cv. Golden acre; cv. American Special Ball Mead	—	LDGP
	<i>B. rapa</i> L.	—	M, Mot, CL
	<i>Iberis amara</i> L.	Y, NS, SL	—
	<i>Matthiola incana</i> R. Br.	—	VC, M, Mot
	<i>Raphanus sativus</i> L.	—	LD, CL, LAR, LDGP
Cucurbitaceae	<i>Coccinia indica</i> Wight & Aran.	—	M, LAR
	<i>Luffa aegyptica</i> Mill.	—	VC, M
	<i>Momordica charantia</i> L.	—	Y, LDGP, LD
	<i>Trichosanthes anguina</i> L.	—	VC, M, YP
Labiatae Leguminosae	<i>Salvia officinalis</i> L.	—	M, LAR
	<i>Cyamopsis tetragonoloba</i> L.	—	M, CL, LD, Mot, LAR
	<i>Phaseolus lunatus</i> L. cv. Lobia Improved Black Seed	RLL	—
	<i>Phaseolus lunatus</i> L. cv. Lobia Pusa Barasati	RLL	—
	<i>Trigonella foenum-graecum</i> L.	BLL	—
	<i>Vicia faba</i> L. cv. The Sutton	RLL	—
Malvaceae	<i>Hibiscus esculentus</i> L. cv. Sutton Makhmali	—	Y, LDGP, LAR
	<i>Hibiscus esculentus</i> L. cv. Long Green; cv. Secen Dhari	—	Y, LDGP, LAR
	<i>Sida cardifolia</i> L.	—	VC, M
Papaveraceae	<i>Eschscholzia californica</i> Cham. cv. Ivory White	—	M
Polemoniaceae	<i>Phlox drummondii</i> Hook	—	M, LAR, LC
Solanaceae	<i>N. megalosiphon</i> Heurek and Mueller	NLL	M, LD, CL, LAR
	<i>N. tabacum</i> cv. GT-4	—	LDGP, LAR, LD
	<i>N. tabacum</i> cv. Lime Green	—	VC, M
	<i>N. tabacum</i> cv. NC-95	—	LDGP, LAR
	<i>N. tabacum</i> cv. Sensation Mixed	—	LD
	<i>N. tabacum</i> cvs Anand 3, Harrison's	NLL	—
	<i>N. tabacum</i> cvs Bhopali Pakra, CTTRI-Special	NLER	—
Umbelliferae	<i>Ammi majus</i> L.	—	LD, St, M
Verbenaceae	<i>Verbena hybrida</i> Voss.	—	LAR, LC, M

Stability of virus in plant sap

The virus in crude sap from infected *N. glutinosa* leaves lost its infectivity after heating at 65 °C for 10 min and at a dilution of 10^{-5} . It remained active for 72 h when stored at room temperature (20 ± 5 °C) and 144 h at 10 °C. The maximum infectivity of virus was found in sap obtained 13 days after inoculation in *N. glutinosa*. In infected turnip leaves dried and stored over calcium chloride, the virus was still infectious after two months.

Purification, sedimentation and Buoyant density

The virus was successfully purified and sedimented as a single peak, suggesting that the preparation was homogenous and contained a single virus component. The sedimentation coefficient was calculated as 118 S.

The virus particles in a purified preparation banded as a single component with a buoyant density of 1.32 g/cm³.

The purified preparation gave a UV-spectrum typical of nucleoprotein. Maximum and minimum absorbance was recorded at 258 and 242 nm respectively. The amount of nucleic acid in the virus was calculated to be 5–6 % by interpolation of the observed data A₂₆₀/A₂₈₀ ratio (1.25) from the graph (GIBBS and HARRISON 1976).

The coat protein of the virus migrated in SDS-PAGE as a single band for which a molecular weight of 25,000 ± 500 daltons was calculated.

Electron microscopy

a) Particle morphology

The purified preparation revealed the presence of flexuous rod shaped particles c. 580 nm in length (Fig. 2) and 13 nm in width.

b) Ultrastructural studies

In ultrathin sections the particles of the virus were found in the mesophyll cells of infected *N. glutinosa* in the form of cytoplasmic inclusion bodies. These were of two forms; virus bundles, and compact and loose banded aggregates of virus particles. Characteristic cytoplasmic laminar bodies were also observed.

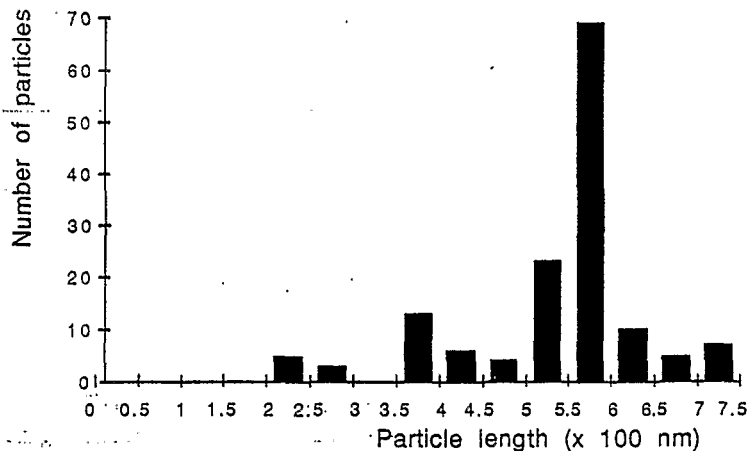


Fig. 2. Distribution of particle length in negatively stained purified preparations (140 particles measured)

The diameter of the virus particles in the inclusions, which were found near the nucleus, was 10.5 nm. The inclusions also contained cytoplasmic ribosomes measuring 20—21 nm in diameter and vacuoles containing an electron-dense material. The banded inclusions were composed of an orderly arrangement of intact virus particles layered with a periodicity of about 520 nm.

Laminar inclusion bodies (beaded sheets) were very distinct; they consisted of laminar sheas about 3—5 nm thick studded on both sides with ribosome-like bodies some 14 nm in diameter. They also were associated with vacuoles filled

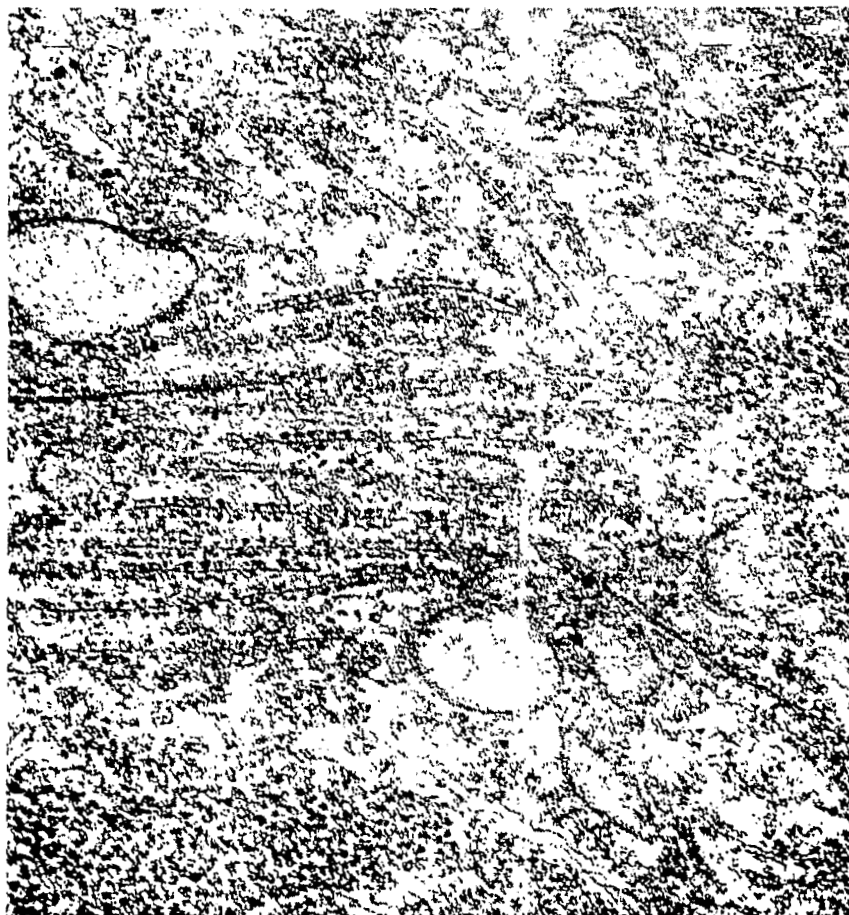


Fig. 3. Beaded sheets in the cytoplasm of infected *Nicotiana glutinosa* leaves ($\times 60\ 000$)

with fibrous material, and a few virus particles were also present near the laminar sheath (Fig. 3). They thus closely resembled to beaded sheet structures reported for PVX but not other potexviruses (LESEMANN 1988, FRANCKI *et al.* 1985).

Serology

Tube precipitin tests, carried out with different combinations of antigen and antiserum, produced granular precipitates. The titer of the antiserum was 1/512, with no reaction against sap from healthy plants.

Both the purified virus preparation and crude sap from infected *N. glutinosa* plants reacted specifically with the antiserum prepared against the virus in the Ouchterlony double diffusion tests. The virus showed a strong reaction in double

diffusion tests with potato virus X (PVX) antiserum but no reaction with antisera of potato Y, potato virus S, potato aucuba mosaic virus, cymbidium mosaic virus, white clover mosaic virus, Narcissus mosaic virus and papaya mosaic virus (Fig. 4).

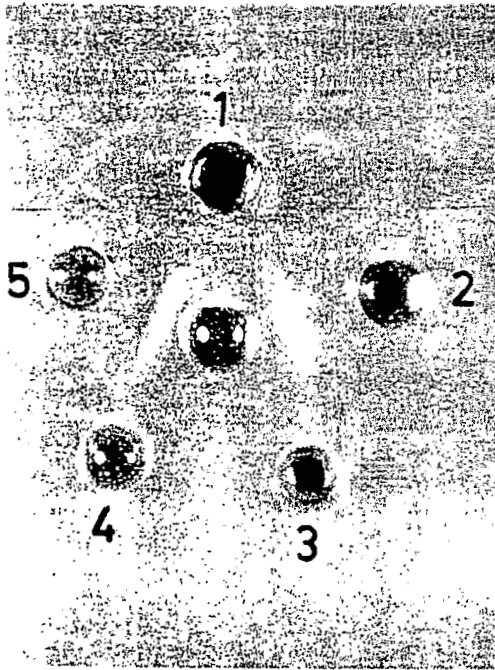


Fig. 4. Double diffusion test in agar with Aligarh strain of PVX. The central well contained purified preparation of the virus and peripheral wells were: 1 - narcissus mosaic virus antiserum, 2 = PVX antiserum, 3 = Cymbidium mosaic virus antiserum, 4 = potato aucuba mosaic virus antiserum, 5 = PVX Aligarh strain antiserum (homologous)

Discussion

The present virus isolate infecting turnip in Aligarh (India) strongly resembles PVX in some of its host reactions (for example the production of necrotic lesions with red haloes on inoculated leaves of *Gomphrena globosa*; mosaic and vein clearing in *Datura stramonium*; necrotic local lesions on *Nicotiana tabacum* varieties and mosaic mottling on some *Nicotiana* species), and is also similar to PVX in biological and biophysical properties, including sedimentation coefficient and the molecular weight of the coat protein (BERCKS 1970). In addition, strong evidence in favour of it being a potexvirus comes from its particle morphology (size ca. 580×13 nm) and the presence of lamellar inclusion bodies. Its relationship with PVX is confirmed by the positive serological reaction with PVX antiserum in Ouchterlony gel double diffusion tests.

There is no known turnip virus which resembles the present virus isolate and hence we conclude that the virus is new to turnip. We have been unable to find any previous report of PVX from turnip; indeed the plant has hitherto been considered insusceptible to this virus (JOHNSON 1930); moreover, in a recent publication (VARMA 1988), turnip does not figure as a host for PVX. We, therefore, propose to call it the Aligarh strain of PVX, infecting turnip.

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