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Pea symptomless virus, a newly recognized strain of red clover mottle virus

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Abstract

A virus contaminating a culture of pea enation mosaic virus was isolated and studied. The virus, tentatively named pea symptomless virus (PSV), was not aphid-borne but was readily transmitted by sap inoculation of Amaranthaceae, Chenopodiaceae and Leguminosae. Purified preparations of PSV contained isometric particles 26 nm in diameter, which sedimented as three components with sedimentation coefficients of 55, 94 and 118S, respectively, and contained ribonucleic acid with a molar base content of G 22%, A 26%, C 17%, U 34%.

PSV is a member of the cowpea mosaic group of plant viruses, is closely related to red clover mottle broad bean stain, and pea green mottle viruses, and protects plants against infection with red clover mottle virus. PSV, the viruses of broad bean stain, red clover mottle and pea green mottle, and possibly MF-virus, probably constitute a subgroup of the cowpea mosaic virus group and may even considered to be members of a single virus species.

Introduction

The virus to be described in this report was found as a contamination in a culture of pea enation mosaic virus (PEMV), maintained for several years in peas by a Dutch plant breeder. The virus was first detected in purified preparations of this PEMV isolate, which contrary to expectations contained both 'full' and 'empty' isometric particles, and produced three boundaries in analytical centrifugation. PEMV contains two sedimenting components only (Izadpanah and Shepherd, 1966) and empty isometric particles have not been observed (Bozarth and Chow, 1967).

Incubation of a purified preparation with PEMV antiserum gave an inoculum free of PEMV; inoculated pea plants remained symptomless and extracts of these plants contained 'full' and 'empty' isometric particles, and failed to react with PEMV antiserum in the Ouchterlony agar double-diffusion test.

The virus was identified as a member of the cowpea mosaic virus (CPMV) group and is closely related to red clover mottle virus (RCMV). Unlike RCMV, however, the virus to be described induces no symptoms in pea plants; therefore, it has been tentatively designated as pea symptomless virus (PSV). Some of its properties are described in this report.

Materials and methods

Using inoculum containing 1 mg of virus/ml various species of plants were tested for

their susceptibility to PSV. After 2–4 weeks, all inoculated plants were checked for infection by back inoculation to peas (*Pisum sativum* 'Koroza'). After two weeks these plants were tested for the virus with antiserum prepared against PSV.

To purify the virus leaves from plants inoculated 12–14 days previously were ground 1 g/2 ml in a Waring blendor in 0.1 *M* phosphate buffer (pH 7.0). After filtration through cheese cloth the sap was clarified at 10,000 *g* for 10 min and the supernatant fluid was frozen overnight. After thawing and clarification, polyethylene glycol (MW = 6,000) and 0.2 *M* NaCl were added to 4% w/v and 0.2 *M* respectively. The precipitate was collected by centrifugation at 10,000 *g* for 20 min and the sediment resuspended in 0.1 *M* phosphate buffer.

After clarification at 10,000 *g* for 10 min the supernatant fluid was centrifuged at 140,000 *g* for 90 min and the pellet resuspended in 0.01 *M* phosphate buffer, pH 7.0. This procedure gave pure preparations as judged by electron microscopy and analytical ultracentrifugation.

RCMV was purified by a similar procedure from pea plants inoculated 9 days previously. Instead of 4% polyethylene glycol, 6% was added to precipitate this virus after thawing and clarification.

The base composition of the nucleic acids was estimated by a chromatographic procedure similar to that described by Knight (1963). RNA was extracted from the viruses as described by van Kammen (1967).

To determine the relationships between PSV and some members of the CPMV group, serological tests were done by micro-precipitin and agar gel diffusion tests. The antisera used were kindly supplied by workers in this and other institutes (Table 3). An antiserum against PSV was prepared by injecting rabbits intramuscularly on three occasions at two week intervals with purified virus emulsified with Freund's incomplete adjuvant.

Results

Host range. PSV induced symptomless infections in the following species and cultivars: *P. sativum* 'Cobri', 'Dick Trom', 'Kebby', 'Kelvedon Wonder', 'Koroza', 'Rondo' and 'Zoete Eminent', *Trifolium incarnatum*, *T. rubens*, *Trigonella caerulea*, *T. foenumgraecum*, *Vicia benghalensis*, *V. orobus*, *V. pannonica*, *V. pisiformis*, *V. sativa*, *V. tenuifolia* and *V. unijuga*. The virus was recovered from all these species and cultivars.

PSV induced necrotic local lesions in *Gomphrena globosa* and local chlorotic lesions in *Chenopodium album*, *C. amaranticolor* and *C. quinoa*. Severe systemic mottling and leaf deformation developed on *C. quinoa* about two weeks after inoculation. A faint green systemic mottling developed on *V. narbonensis* about 10 days after infection and diffuse chlorotic mottling appeared on *P. jamardii* after 7 days. Virus could also be recovered from these plants. The following species produced no symptoms and contained no virus: *Arachis hypogaea*, *Cassia acutifolia*, *C. angustifolia*, *Cucumis sativus*, *Echinocystis lobata*, *Glycine max*, *Glycyrrhiza glabra*, *Lathyrus heterophyllus*, *L. montanus*, *L. odoratus*, *L. pratensis*, *Lotus corniculatus*, *L. uliginosus*, *Lupinus luteus*, *Lycopersicon esculentum*, *Medicago sativa*, *Melilotus officinalis*, *Nicotiana glutinosa*, *N. tabacum*, *Petunia hybrida*, *Physalis floridana*, *Robinia pseudoacacia*, *Securigera suffruticosa*, *Tetragonolobus purpureus*, *Vigna unguiculata* and *Zinnia elegans*.

The sensitivity of a number of plant species to PSV and RCMV was compared

Table 1. Comparative host range and symptomatology of pea symptomless virus (PSV) and red clover mottle virus (RCMV).

Plant species	PSV	RCMV
Chenopodium amaranticolor	local chlorotic spots	local chlorotic spots
C. quinoa	local chlorotic spots, followed by a systemic mottling and leaf deformation	similar but more severe than PSV
Pisum jamardii	systemic diffuse chlorotic mottling	systemic mosaic, followed by top necrosis and death of the plant
Vicia narbonensis	faint green mottling	same as above
Trigonella foenum-graecum	no symptoms	deformation of top leaves followed by top necrosis and death of the plant
V. faba	as above	as above
V. sativa	as above	as above
V. pannonica	as above	as above
V. benghalensis	as above	as above

Tabel 1. Vergelijking van de symptomen die het 'pea symptomless virus' (PSV) en het rode-klavervlekkenvirus (RCMV) op een aantal plantesoorten veroorzaken.

(Table 1). Both viruses induced essentially similar symptoms in *C. amaranticolor* and *C. quinoa*. But RCMV invariably caused more severe symptoms than PSV in *P. jamardii*, *V. narbonensis*, *T. foenum-graecum*, *V. faba*, *V. sativa*, *V. pannonica* and *V. benghalensis;* these differences allowed the viruses to be readily distinguished.

The two viruses seem to have nearly identical host ranges. Only *V. unguiculata* and *P. vulgaris*, which are susceptible to RCMV (Bos and Maat, 1965), were found to be immune to PSV.

Transmission. The virus was readily transmitted by sap inoculation. Aphids (*Myzus persicae* and *Acyrthosiphon pisum*) failed to transmit the virus when allowed either to make short (periods of seconds and minutes) or long (periods of days) acquisition and infection feeding periods. Moreover, these aphid species failed to acquire PSV from plants containing both PEMV and PSV.

Properties of PSV in crude plant sap. The infectivity of sap from infected pea plants was lost after 10 min at 65° but not at 60 °C. The virus retained its activity for at least 3 years in leaf material stored at −18 °C and in pure preparations kept at the same temperature for more than one year.

Properties of purified preparations. Electron microscopy showed that PSV has 'full' and 'empty' polyhedral particles c. 26 nm diameter (Fig. 1). They closely resembled those of members of the CPMV group, such as RCMV and CPMV.

In the analytical centrifuge, purified preparations of PSV produced three boundaries with sedimentation constants of 55, 94, and 118S.

The middle component always accounted for the largest peak followed by the bottom component. The amount of the top component was invariably smaller than that of the bottom component (Fig. 2). The three components in PSV preparations

Fig. 1. Electron micrograph showing PSV particles mounted in neutral PTA.

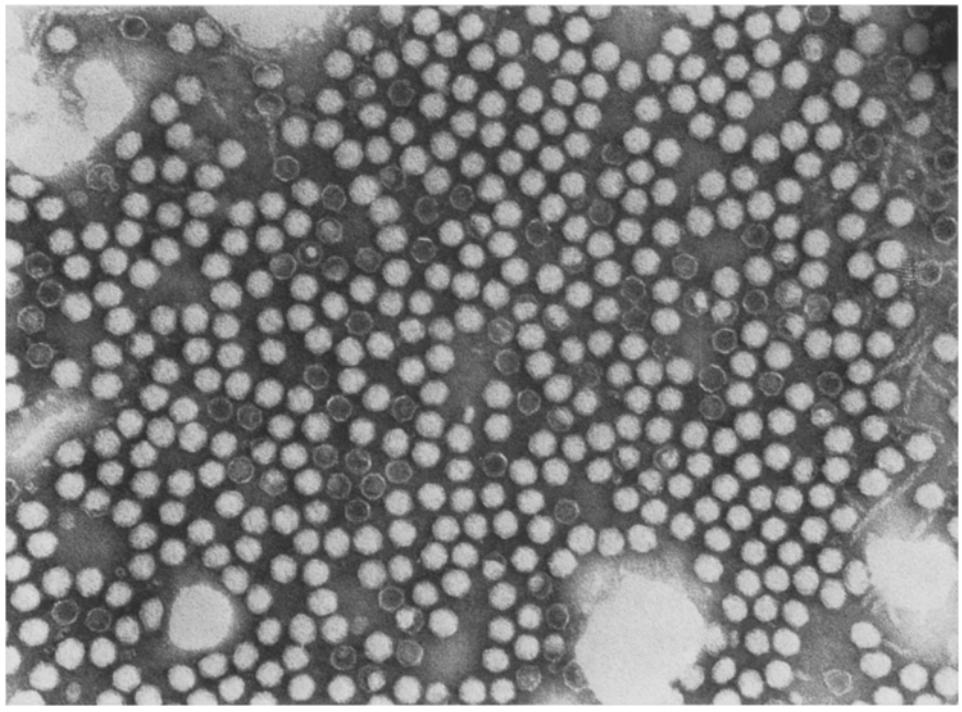

Fig. 1. Elektronenmicroscopische opnamen van 'pea symptomless virus' (PSV) dat met neutraal wolfraamzuur werd gecontrasteerd.

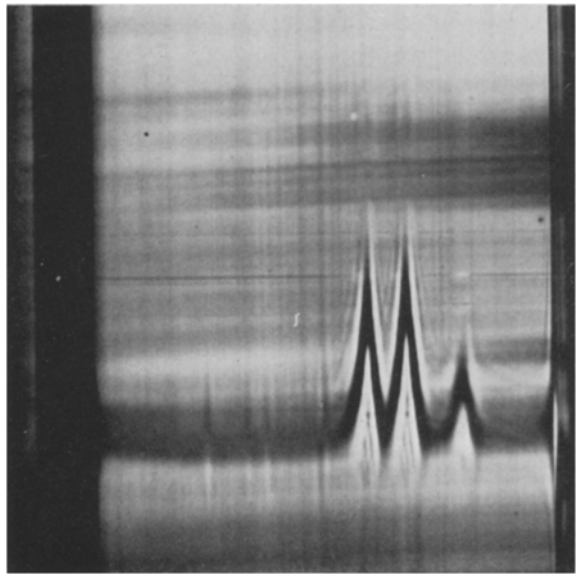

Fig. 2. Schlieren diagram of PSV. Sedimentation is from left to right. Speed 31,410 rev/min (Spinco model E). Photograph taken 9 min after reaching speed.

Fig. 2. Sedimentatiediagram van 'pea symptomless virus' (PSV). De sedimentatie verloopt van rechts naar links. Snelheid 31.410 (Spinco Model E). De foto werd gemaakt 9 minuten nadat de centrifuge de afgestelde snelheid had bereikt.

Table 2. Molar base ratio of PSV and other members of the cow-pea mosaic virus group.

	Moles (%)				Source
	Guanine	Adenine	Cytosine	Uracil	
PSV[1]	22.1	26.5	17.2	33.8	this study
RCMV[2]	25.1	25.3	19.8	29.8	this study
RCMV[3]	20.3	29.0	20.4	30.2	Gibbs et al. (1968)
BBSV[4]	23.3	26.5	18.4	31.9	Gibbs et al. (1968)
CPMV[4]	20.7	28.4	19.3	31.6	van Kammen and van
CPMV[5]	22.9	28.5	17.2	31.4	Griensven (1970)
SqMV[6]	23	32	16	30	Mazzone et al. (1962)

[1]Pea symptomless virus [2]Red clover mottle virus [3]Broad bean stain virus [4]Cowpea mosaic virus [5]Squash mosaic virus

Tabel 2. De verhouding van de basen in het ribonucleïnezuur van het 'pea symptomless virus' (PSV) en andere leden van de 'cowpea mosaic virus' groep.

were separated by centrifugation in sucrose density gradients and had the same external morphology.

The sedimentation coefficients of the components of PSV suggest (Reichmann, 1965) that the two faster sedimenting components contain 26 and 36% nucleic acid, respectively. The nucleic acid of PSV had a base composition (Table 2), which resembled those of two strains of CPMV, RCMV as determined by Gibbs et al. (1968), broad bean stain virus (BBSV) and squash mosaic virus (SqMV). The base composition of RCMV found in this study differs somewhat from the ratio given by Gibbs et al (1968).

Serology. Our tests gave evidence that PSV and RCMV are closely related to each other (Table 3). Both viruses are also related to BBSV and pea green mottle virus (PGMV) (Valenta et al., 1969). The virus was distantly related to CPMV strains used in our laboratory. An antiserum prepared by Hollings against van Kammen's yellow strain of CPMV, showed a closer relation to PSV and RCMV. PSV and RCMV are even distantly related to SqMV, bean pod mottle virus (BPMV), broad bean mottle virus (BBMV), true broad bean mosaic virus (TBBMV) and PEMV.

Cross-protection tests. Pea plants (*P. sativum* 'Koroza') were inoculated with PSV. Groups of 10 plants were challenged with RCMV 3, 6 and 9 days after the inoculation with PSV. Control plants were also inoculated with RCMV 3, 6 and 9 days after a first inoculation with water; all these control plants (30 in total) clearly showed RCMV symptoms 9 days after the challenge inoculation. Two weeks after the challenge inoculation RCMV symptoms appeared respectively on 10, 4 and 0 of the plants inoculated with PSV. The plants which were apparently not infected with RCMV proved to be free of RCMV in back inoculations to peas. These results suggest that PSV could confer partial protection of pea plants against RCMV when the infection of PSV is 6 days old. The protection is complete 9 days after the first inoculation.

Table 3. Titres of antisera to PSV and other members of the CPMV group, in tests with PSV and RCMV.

Antiserum prepared against	Antiserum titres obtained with			Source[2]
	PSV	RCMV	RCMV[1]	
PSV[3]	256	128		Present authors
RCMV[3]	128	256	256	Maat
PGMV[4]	32	8	32	Valenta
BBSV[3]	64	16	128	Harrison
CPMV 'yellow'[3]	4	1	0	van Kammen
CPMV 'severe'[3]	4	1		van Kammen
CPMV 'Littlehampton'[3]	64	0		Hollings
SqMV[3]	0	0	0	Hollings
BPMV[5]	0	0	0	Hollings
BBMV[6]	0	0		Hollings
TBBMV[7]	0	0	0	Hollings
PEMV[8]	0	0		Present authors

[1]Titres found by Gibbs et al. (1968) when RCMV was tested against different members of the cowpea mosaic group.
[2]The authors kindly express their thanks to the suppliers of the sera.
[3]See footnotes Table 2.
[4]Pea green mottle virus.
[5]Bean pod mottle virus.
[6]Broad been mottle virus.
[7]True broad been mosaic virus.
[8]Pea enation mosaic virus.

Tabel 3. Titers van antisera tegen het 'pea symptomless virus' (PSV) en andere leden van de 'cowpea mosaic virus' groep (CPMV), zoals die werden bepaald in toetsen met PSV en rode-klavervlekkenvirus (RCMV).

Discussion

On the basis of morphological, physical and antigenic properties PSV (cryptogram: R/1 :*/36+*/26:S/S:S/*) clearly belongs to the cowpea mosaic virus (CPMV) group, which contains bean pod mottle, broad bean stain, cowpea mosaic, pea green mottle, radish mosaic, red clover mottle, and squash mosaic viruses (Gibbs et al., 1968; Valenta et al., 1969).

Our results show that PSV is closely related to RCMV and less closely to BBSV and PGMV. Gibbs et al. (1968), Valenta and Gressnerovà (1969), and Valenta et al. (1969) have already pointed out the close relationship of these viruses. Another virus, designated MF (mosaïque de la Fève) virus (Devergne and Cousin, 1966) may also belong to this group. Devergne and Cardin (1968) studied the relationships between MF and the Dutch and English strains of RCMV. The relationship between the Dutch strain and MF was less close than that between the English strain and MF. Since the two RCMV strains were more closely related to each other than to MF virus, these authors concluded that the two strains and MF virus belonged to two distinct serotypes. Valenta and Gressnerovà (1969) concluded that MF was closely related to BBSV and PGMV.

The other members of the CPMV group show either a more distant or no serological relationship to PSV. The existance of a close serological relationship between PSV, BBSV, RCMV, PGMV and MF suggests that all these viruses either may constitute a subgroup within the CPMV group or are strains of a single virus. A more thorough serological study of this group should be made. If they are considered a subgroup it should be designated the RCMV subgroup for priority reasons.

The antigenic relationship between PSV and the Dutch strain of RCMV is so close that we are inclined to consider these entities as distinct strains of a single virus. The strong cross protection between PSV and RCMV in peas also supports this conclusion. Until the relationships between these two viruses and BBSV, MF and PGMV have been studied in more detail, and because PSV clearly differs from RCMV in symptomatology, we prefer retaining a separate name for PSV.

Samenvatting

De karakterisering van een nieuw virus dat nauw verwant is aan het rode-klavervlekkenvirus

Een virus dat als verontreiniging voorkwam in een cultuur van het erwte-enatiemozaïekvirus werd geïsoleerd en bestudeerd. Het virus dat voorlopig met de naam 'pea symptomless virus' (PSV) wordt aangeduid, gaat met sap over en wordt niet door de bladluizen *Myzus persicae* en *Acyrthosiphon pisum* overgedragen.

Plantesoorten uit de families der Amaranthaceae, Chenopodiaceae en Leguminosae (Tabel 1) zijn vatbaar voor dit virus. Gezuiverde preparaten van PSV bevatten isometrische deeltjes met een diameter van 26 nm (Fig. 1) en bestonden uit drie fracties (met sedimentatieconstanten van 118, 94 en 55S (Fig. 2). Voor de basenverhouding in het RNZ werd 22% guanine, 26% adenine, 17% cytosine en 34% uracil (Tabel 2) gevonden.

Het beschreven virus is serologisch zeer nauw verwant met het rode-klavervlekkenvirus (RCMV) en wat minder verwant met andere leden van de 'cowpea mosaic virus' (CPMV) groep, zoals het 'broad bean stain virus' (BBSV), en 'pea green mottle virus' (PGMV). De symptomen die PSV en RCMV veroorzaakten werden vergeleken (Tabel 1); RCMV veroorzaakte in alle gevallen heviger symptomen.

PSV is in staat om erwteplanten volledig tegen RCMV te beschermen indien de planten 9 dagen van te voren met eerstgenoemd virus geïnoculeerd waren. De auteurs concluderen uit hun werk dat PSV met BBSV, PGMV en RCMV een sub-groep binnen de CPMV-groep vormen, waartoe ook het MF-virus dat in Frankrijk is gevonden, zou kunnen worden gerekend.

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