

# PHYSICAL AND CHEMICAL PROPERTIES OF SEVERAL PLANT VIRUSES

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## ABSTRACT

Some of the physical and chemical properties of several characteristic plant viruses have been analysed recently and the results are presented here. The viruses used are as follows: melon necrotic spot virus, a spherical virus composed of a single particle component and one non-segmented, single-stranded RNA; mulberry ringspot virus, a spherical virus composed of three particle components and two-segmented, single-stranded RNA; rice gall dwarf virus, a spherical virus composed of a single particle component and twelve-segmented, double-stranded RNA; soybean chlorotic mottle virus, a spherical virus composed of a single particle component and one non-segmented, double-stranded DNA.

The comparative properties of the viruses and the methodology applied to the analyses are discussed.

## Introduction

For the accurate classification and identification of plant viruses, it is necessary to analyse some physical and chemical properties of the viruses, such as the number of particle components (single- or multi-components), the species of the nucleic acid (RNA or DNA; single- or double-stranded), the number and molecular weight of the segments of the nucleic acid, nucleic acid content of the virion, molecular weight of the capsid protein(s), etc., as well as the biological, serological and morphological properties of the viruses. Recently, we analysed some of the physical and chemical properties of several characteristic plant viruses and the results are presented here.

## Melon necrotic spot virus

Melon necrotic spot virus (MNSV) is a virus with isometric particles *ca.* 30nm in diameter, which is transmitted in soil by the chytrid fungus *Olpidium radicale* (= *O. cucurbitacearum*), and readily transmitted by mechanical inoculation to several species of Cucurbitaceae. The virus occurs naturally only in greenhouse melons and cucumbers, and causes significant decrease in yield. The virus was first recorded in Japan (Kishi, 1960), and presently it is found also in the USA, the Netherlands and UK (Hibi and Furuki, 1985).

As some of the biological properties of MNSV appear to be very similar to those of cucumber necrosis virus (CNV) reported already in Canada (Dias and Mckeen, 1972), some of the physical and chemical properties of MNSV and CNV, as well as serological relationships, were compared (Hibi *et al.*, 1980).

The two viruses had no serological relationships with each other and differed in their buoyant density in CsCl (1.34 g/ml for MNSV; 1.35 g/ml for CNV) and in the sedimentation coefficient determined on preparations with  $A_{260} = 5$  (123 S for MNSV; 128 S for CNV). The viral nucleic acids consisted both of one molecule of single-stranded RNA and differed slightly from each other in their molecular weights ( $1.5 \times 10^6$  for MNSV;  $1.6 \times 10^6$  for CNV). These results revealed that MNSV is a different virus from CNV, although the two viruses belong to the same virus group.

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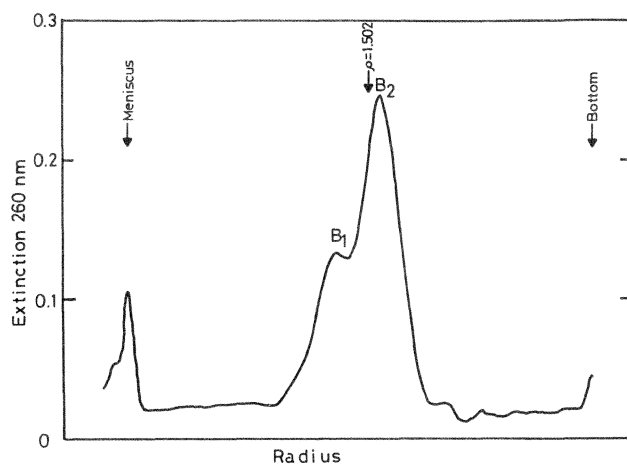
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### Mulberry ringspot virus

Mulberry ringspot virus (MRSV) is a virus with isometric particles *ca.* 25nm in diameter, which is transmitted in soil by the nematode *Longidorus martini*, and readily transmitted by mechanical inoculation to several species of Moraceae, Leguminosae, and Chenopodiaceae. The virus occurs naturally on mulberry, and causes mosaic and ringspot symptoms on the leaves. It was found only in Japan (Tsuchizaki *et al.*, 1971; Tsuchizaki, 1975).

Although MRSV was known as a nepovirus, the genome composition of the virus had not been determined and such properties were thus analysed (Hibi *et al.*, 1984).

MRSV is composed of two particles components, M (93S) and B (122S), in sucrose density gradient centrifugation, but the B component was shown to be separated into further two components, B<sub>1</sub> (1.497 g/ml) and B<sub>2</sub> (1.504 g/ml), in CsCl isopycnic analytical ultracentrifugation (Fig. 1), although it was impossible to fractionate B<sub>1</sub> and B<sub>2</sub> separately. The nucleic acid of the virus showed a hyperchromicity with HCHO and a buoyant density of 1.633 g/ml in Cs<sub>2</sub>SO<sub>4</sub>, suggesting that the nucleic acid is single-stranded RNA. RNA isolated from the M component consisted of a single molecule, RNA-2 (M.W.  $1.4 \times 10^6$ ), but RNA from the B component (B<sub>1</sub> + B<sub>2</sub>) was composed of two types of molecules, RNA-1 (M.W.  $2.6 \times 10^6$ ) and RNA-2 (M.W.  $1.4 \times 10^6$ ). The M component had no infectivity, but the B component showed infectivity in the absence of the M component. Although the infectivity assay of each RNA segment was unsuccessful, the results suggested that (1) the genome RNA of MRSV is composed of two segments, RNA-1 and RNA-2, (2) M component contains one molecule of RNA-2, (3) B<sub>1</sub> component contains one molecule of RNA-1, and (4) B<sub>2</sub> component contains two molecules of RNA-2. The genome RNA composition of MRSV appeared to be very similar to that of raspberry ringspot virus, a type virus of nepovirus group (Murant, 1978).



**Fig. 1** Sedimentation profile of B component of MRSV after isopycnic ultracentrifugation in CsCl.

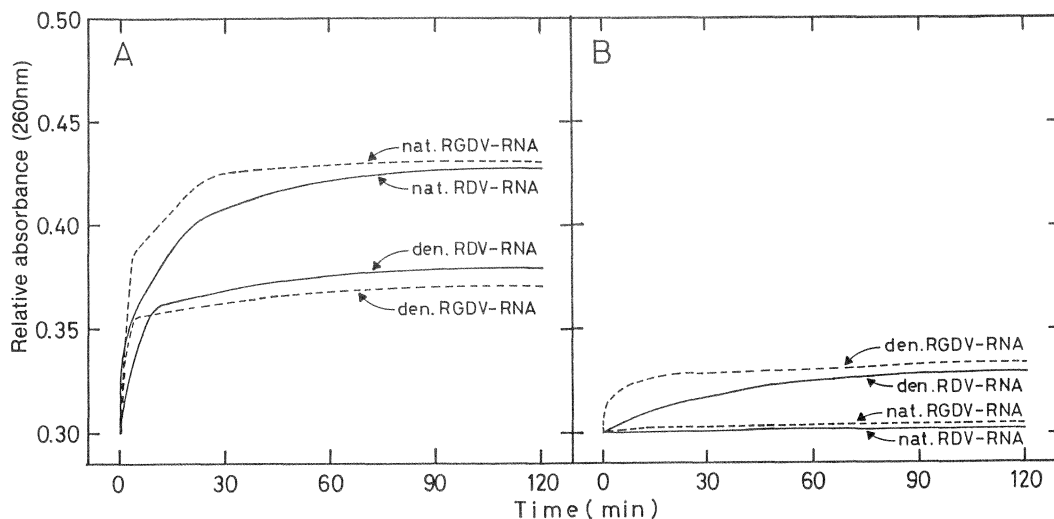
### Rice gall dwarf virus

Rice gall dwarf virus (RGDV) is a virus with isometric particles *ca.* 65 nm in diameter, which is transmitted in a persistent manner by five species of leafhoppers, and causes severe stunting and gall formation on rice plants (Omura *et al.*, 1980; Inoue and Omura, 1982). No serological relationships were found between RGDV and eight other viruses from the genera Phytoreovirus and Fijivirus (Omura *et al.*, 1982). The virus was detected in Thailand in 1980.

As some of the biological and morphological properties of RGDV appear to be very similar to those of rice dwarf virus (RDV) reported already in Japan (Iida *et al.*, 1972), some properties of the nucleic acids extracted from RGDV and RDV were compared (Hibi *et al.*, 1984).

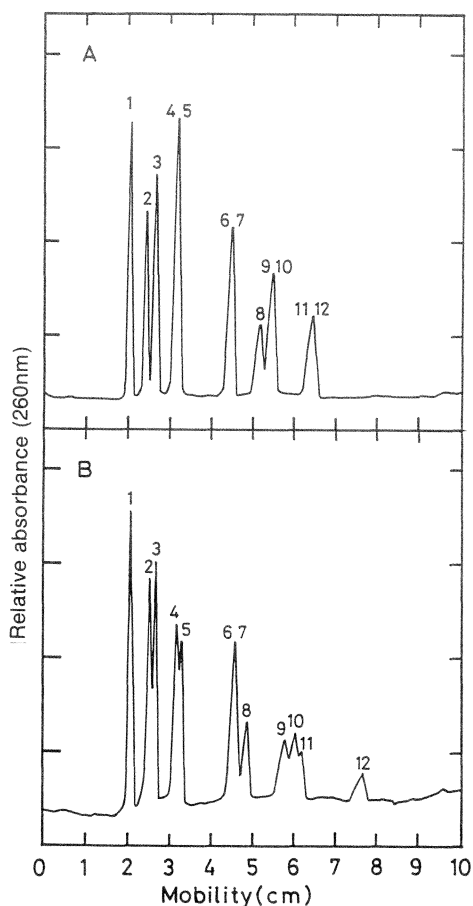
Nucleic acid of RGDV was identified as double-stranded RNA because it (1) was susceptible to RNase A in  $0.1 \times$  SSC but not in  $1 \times$  SSC (Fig. 2), (2) was resistant to nuclease S1, (3) showed no hyperchromicity in UV absorption after treatment with HCHO at  $37^\circ\text{C}$ , (4) showed a sharp thermal transition in UV absorption in  $0.01 \times$  SSC and (5) had a buoyant density of  $1.596 \text{ g/ml}$  in  $\text{Cs}_2\text{SO}_4$ .

Electrophoresis in polyacrylamide slab gel revealed that RGDV RNA was composed of 12 segments with a total MW of about  $16.9 \times 10^6$ . Co-electrophoresis of RNA from RGDV and RDV demonstrated that the electrophoretic mobilities of the seven larger segments from the two viruses were similar but that the five smaller segments differed in this respect (Fig. 3). These results confirm that RGDV is a new virus and a third member of the genus Phytoreovirus.



**Fig. 2 Comparison of the kinetics of digestion of RGDV RNA and RDV RNA by RNase A as measured by the increase in absorbance at 260 nm.**

About  $15 \mu\text{g/ml}$  of native (nat.) or denatured (den.) nucleic acid was incubated at  $25^\circ\text{C}$  in either  $0.1 \times$  SSC (a) or  $1 \times$  SSC (b) with  $1 \mu\text{g/ml}$  (a) or  $10 \mu\text{g/ml}$  (b) RNase.



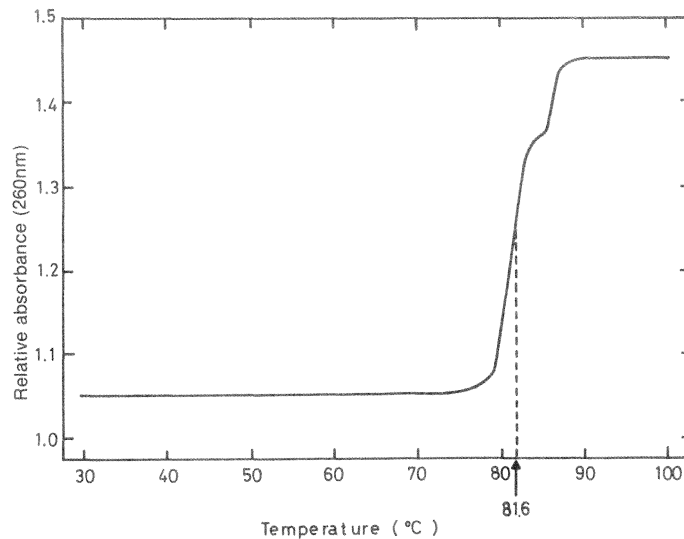
**Fig. 3** Electrophoregrams of double-stranded RNA segments from RDV (a) and RGDV (b) in 2.5% polyacrylamide gel. Each lane of the gel was scanned at 260 nm.

### Soybean chlorotic mottle virus

Soybean chlorotic mottle virus (SoyCMV) is a virus with isometric particles *ca.* 50 nm in diameter, which is transmitted by mechanical inoculation, but not by aphids to four species of Leguminosae, and causes chlorotic mottling and stunting on plants (Iwaki *et al.*). SoyCMV was not related serologically to cauliflower mosaic virus (CaMV) or carnation etched ring virus (CERV), two caulimoviruses. It was found in Japan in 1984.

As some of the biological and morphological properties of SoyCMV suggested that it might be a new caulimovirus, some properties of the nucleic acids extracted from SoyCMV and CaMV were compared (Hibi *et al.*, 1983).

Nucleic acid OF SoyCMV was identified as open circular double-stranded DNA because it (1) showed a sharp thermal transition in UV absorption with a  $T_m$  of 81.6°C in  $1 \times$  SSC (Fig. 4), (2)



**Fig. 4 Thermal denaturation kinetics of SoyCMV DNA in  $1 \times \text{SSC}$ .**

had a buoyant density of 1.688 g/ml in CsCl, (3) formed a single band after equilibrium density gradient centrifugation in CsCl in the presence of EtBr, (4) showed blue fluorescence on staining with 4', 6-diamidino-2-phenylindole (DAPI) and (5) showed a typical circular form under the electron microscope.

Electrophoresis of SoyCMV DNA in agarose slab gel produced multiple bands considered to be circular and linear forms of a molecule with an approximate MW of  $5 \times 10^6$ . The DNA contained 30% guanine plus cytosine and was infectious on *Phaseolus vulgaris*.

The restriction map and the hybridization test suggested that the nucleotide sequences of SoyCMV DNA might be completely different from those of CaMV DNA (Hibi *et al.*, 1985). These results confirm that SoyCMV is a new virus of the genus Caulimovirus.

### Discussion

As shown above, physical and chemical characterization of several plant viruses revealed that (1) MNSV is a typical single-component, single-stranded RNA virus, (2) MRSV is a multi-component, single-stranded RNA virus, (3) RGDV is a double-stranded RNA virus and (4) SoyCMV is a double-stranded DNA virus. The relationships of each virus to the virus groups to which they belong were also elucidated.

For these analyses, several physical and biochemical methods, including analytical ultracentrifugation, thermal denaturation kinetics analysis, nuclease digestion kinetics analysis, gel electrophoresis, etc. were used without radioisotopes.

The difficulty in detecting the viruses for a long time may be ascribed to the very low yields of the newly found viruses.

Therefore, it is necessary to use more sensitive, and, if possible, simple microanalytical methods for the physical and chemical characterization of such viruses. Analytical ultracentrifugation is an example of a suitable microanalytical method for the study of virions and their nucleic acids.

Presently, in addition to conventional biochemical data, some molecular biological data of the

viruses, such as nucleotide sequences and their homology, are essential for the classification, identification and diagnosis of most of the plant viruses. It is also necessary to develop more simple and practical, if possible non radioactive, methods suitable for such molecular biological analyses of plant viruses.

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### Discussion

**Reddy, D.V.R.** (ICRISAT): 1. Did you compare the genome profile of wound tumor virus (WTV) and RGDV? 2. How wide is the host range of the caulimovirus you studied, namely soybean chlorotic mottle virus? Did you determine the buoyant density of the virus particles?

**Answer:** 1. The electrophoretic pattern of the double-stranded RNA appears to be the same. I agree with you that the electrophoretic mobility of RNA may vary with the conditions. 2. The host range of soybean chlorotic mottle virus is much narrower than that of peanut chlorotic leaf streak. We did not determine the buoyant density of the virus particles.