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Morphological Characteristics of Rice Stripe Virus

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SUMMARY

The morphological characteristics of particles of rice stripe virus (RSV) were examined. Each of four components (M1, M2, B and nB) of RSV, isolated by repeated cycles of sucrose density gradient centrifugation, contained circular filaments; the modal lengths were 510 nm for the M1 component, 610 nm for the M2 component, 840 nm for the B component and 2110 nm for the nB component. Each component was associated with a single-stranded species and a double-stranded species of RNA although a fifth component (the T component) did not form a distinct band after the density gradient centrifugation and was associated with circular filaments of 290 nm in length and a ssRNA species.

Rice stripe virus (RSV), maize stripe virus (MStV), rice grassy stunt virus (RGSV) and rice hoja blanca virus form the tenuivirus group (Gingery, 1987). They have filamentous particles, infect plants of the family Gramineae and are transmitted by planthoppers (Koganezawa, 1977; Gingery *et al.*, 1981; Toriyama, 1982; Morales & Niessen, 1983; Hibino *et al.*, 1985).

MStV was reported to be associated with five single-stranded and five double-stranded RNAs (Falk & Tsai, 1984). RSV is associated with four ss- and four dsRNAs (Toriyama & Watanabe, 1989; Ishikawa *et al.*, 1989), and RGSV is associated with four ssRNAs (Toriyama, 1987). Particles of RSV and RGSV were found to be circular filaments (Hibino *et al.*, 1985) of 100 to 1200 nm in length for RSV and 200 to 2400 nm for RGSV. In this paper the shapes and RNA constituents of five isolated components of RSV are described.

The components of RSV were purified following the methods described previously (Ishikawa *et al.*, 1989) and after 10 to 40% sucrose density gradient centrifugation at 88000 *g* for 3·5 h, three bands (designated M, B and nB in order from top to bottom) were obtained (Toriyama, 1982; Ishikawa *et al.*, 1989). Electrophoresis in composite gels (2·0% acrylamide, 0·5% agarose) (Ishikawa *et al.*, 1989) revealed that each band contained three or four sets of ss- and dsRNAs as reported by Toriyama & Watanabe (1989). Three or four cycles of centrifugation through sucrose gradients yielded four components (M1, M2, B and nB), as described previously (Ishikawa *et al.*, 1989). After the last centrifugation, each component was recovered from the centre of each band, suspended in 0·1 M-potassium phosphate buffer pH 7·0 and subjected to an additional cycle of density gradient centrifugation. Electrophoresis in a composite gel showed that particles of each of these highly purified components contained only one set of ss- and dsRNAs, which were then used in further experiments. The M_r values of respective RNAs were reported previously (Table 1) (Ishikawa *et al.*, 1989).

A particle fraction obtained from just above the M band after the first density gradient centrifugation was found to contain an RNA species, smaller than the M1 component and was shown to be single-stranded by incubation with RNase A (10 μg/ml) in 0·3 M-NaCl, 0·03 M-sodium citrate for 1 h at 37 °C. After more than three cycles of density gradient centrifugation this particle fraction was obtained as the top fraction (T). The M_r value of ssRNA in the T fraction was estimated as $0·58 \times 10^6$ (Fig. 1).

Purified component particles (T, M1, M2, B and nB) were mounted on grids and stained with 4% uranyl acetate for electron microscopy (Hibino *et al.*, 1985). Purified particles were also

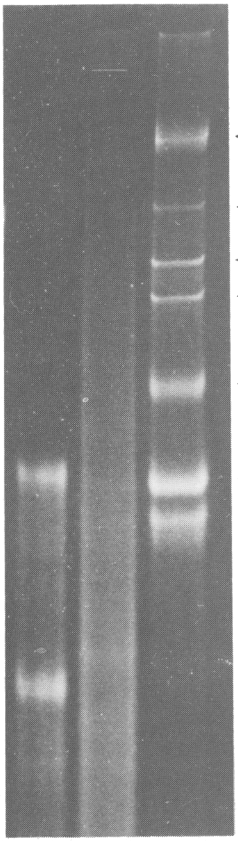

Fig. 1. Electrophoresis of RSV RNAs in a composite gel. The RNAs in lane 1 are 23S and 16S ribosomal RNA of *Escherichia coli*, the RNA in lane 2 was extracted from T component and the RNAs in lane 3 were extracted from the mixture of M1, M2, B and nB components. ss-1 to -5 and ds-1 to -4 indicate the ssRNAs and dsRNAs associated with RSV, respectively.

Table 1. *Relationship between RNA* M_r *and length for each component particle*

	Component particle				
	T	M1	M2	B	nB
Length of particle (nm)	290	510	610	840	2110
Size of RNA ($M_r \times 10^{-6}$)					
ssRNA	0·58	1·0†	1·2†	1·5†	3·1†
dsRNA	ND‡	1·7†	2·1†	2·8†	5·0†
Value of ratio ($\times 10^{-3}$)*					
ssRNA	2·0	2·0	2·0	1·8	1·5
dsRNA	–	3·3	3·4	3·3	2·4

* Value of ratio indicates RNA molecular mass per unit length (nm) in each component.
† RNA M_r values from Ishikawa *et al.* (1989).
‡ ND, Not detected.

mounted on grids and treated with gold-conjugated antibody for labelling, following the method described by Pares & Whitecross (1982). Grids were examined using a JEM-1200 EX electron microscope (JEOL) and the sizes of particles determined on prints at 160000-fold magnification with a graphic calculator. The modal length of tobacco mosaic virus (300 nm) was used as a standard for calibration of the electron microscope. Circular filamentous particles approximately 8 nm in width, as found previously (Ishikawa *et al.*, 1989), were observed in the T, M1, M2, B and nB components (Fig. 2 to 6) but linear filaments were rarely seen in these preparations. The particle lengths of circular filaments in each component are shown in

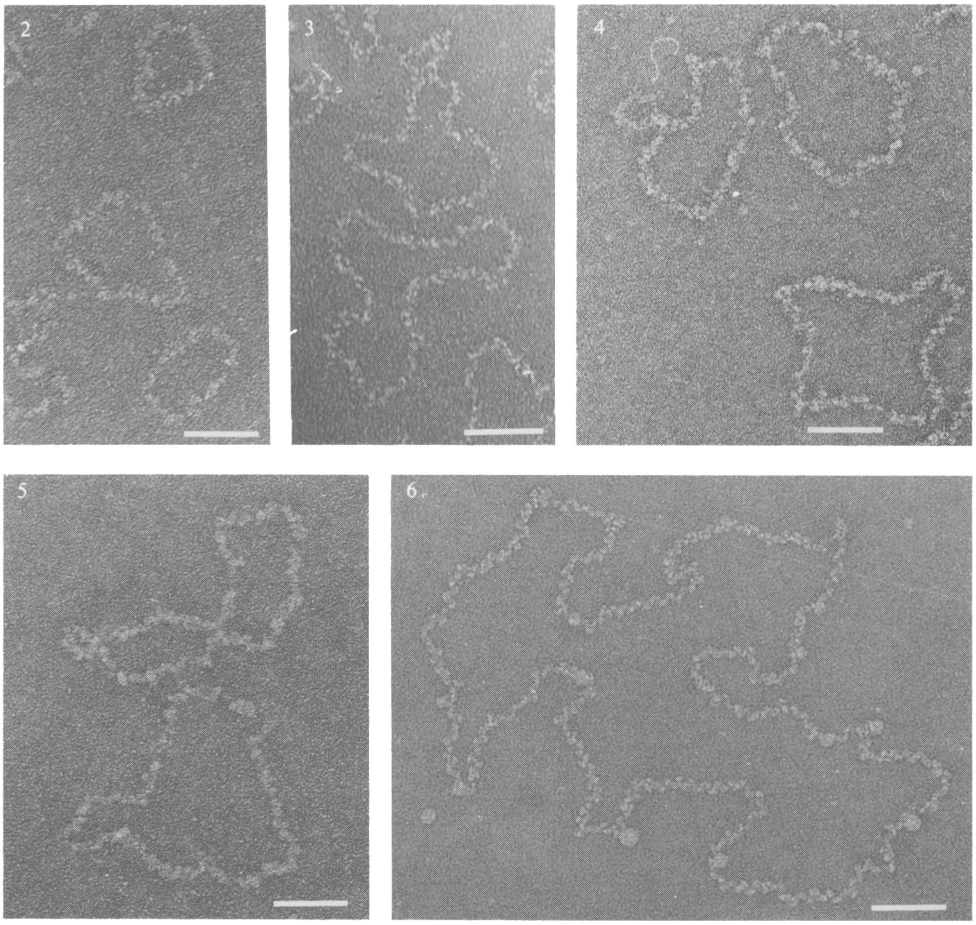

Fig. 2 to 6. Circular filamentous particles observed in each component of RSV (T, M1, M2, B and nB respectively), stained with 4% uranyl acetate. the bar markers represent 100 nm.

Fig. 7(*a*) to (*e*). The modal lengths of T, M1, M2, B and nB component particles were 280, 510, 610, 840 and 2110 nm, respectively.

T component filaments reacted with monoclonal antibodies against RSV in ELISA (Omura *et al.*, 1986) and, like the M1, M2, B and nB component filaments, they reacted in a gold-conjugated antibody labelling test (Fig. 8).

The relationship between RNA M_r and particle length of each component was analysed to assess the structural uniformity of the five components. The ratio was approximately $2{\cdot}0 \times 10^3$ for ssRNAs in the T, M1, M2 and B components and $3{\cdot}3 \times 10^3$ for dsRNAs in the M1, M2 and B components (Table 1); thus, the ratios for both ss- and dsRNAs were consistent except for nB. This inconsistency is unlikely to be due to structural heterogeneity of the components, as they appeared to be similar in their morphology and have the same buoyant density (Toriyama, 1983). Assuming the ratio of RNA M_r and the particle length is constant the M_r of ss- and dsRNA in the nB component is calculated as $4{\cdot}2 \times 10^6$ and $7{\cdot}1 \times 10^6$ which are slightly larger values than those determined previously by electrophoresis (Table 1) (Ishikawa *et al.*, 1989).

RSV is serologically related to MStV (Gingery *et al.*, 1983), which is associated with five sets of ss- and dsRNAs (Falk & Tsai, 1984) and probably also with five components. Association of five component particles with RSV further supports the relationship of the two viruses. This suggests that tenuiviruses are characterized by having five component particles, which are circular filaments of different lengths.

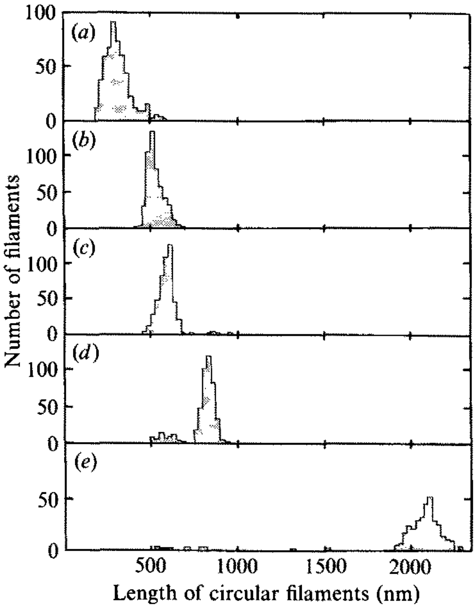

Fig. 7

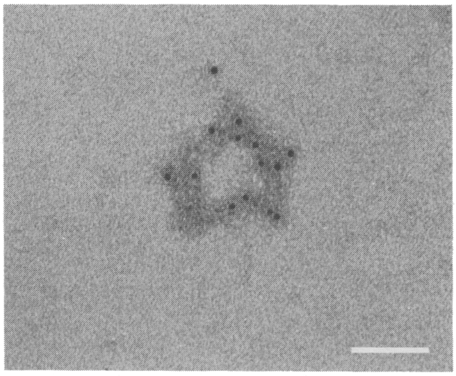

Fig. 8

Fig. 7. Length distribution of circular filamentous particles in the (*a*) T, (*b*) M1, (*c*) M2, (*d*) B and (*e*) nB components of RSV, observed by electron microscopy after staining with 4% uranyl acetate.

Fig. 8. Deposition of gold-conjugated RSV monoclonal antibody on a circular filament of T component. The bar marker represents 100 nm.

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