

Key words: *peanut clump virus*/*RNA species*/*furoviruses*

Genome Properties and Relationships of Indian Peanut Clump Virus

By D. V. R. REDDY, † D. J. ROBINSON, I. M. ROBERTS
AND B. D. HARRISON*

Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, U.K.

(Accepted 25 June 1985)

SUMMARY

RNA extracted from the rod-shaped particles of Indian peanut clump virus (IPCV), strain L, consisted predominantly of two single-stranded species with mol. wt., estimated by gel electrophoresis of glyoxal-denatured samples, of 1.83×10^6 (RNA-1) and 1.35×10^6 (RNA-2). Both RNA-1 and RNA-2 were needed for lesion production in leaves of *Phaseolus vulgaris* cv. Topcrop. Strong nucleotide sequence homologies were detected among three strains of IPCV by nucleic acid hybridization tests. Less strong homologies were found between these Indian isolates and peanut clump virus (PCV) from West Africa. These results, together with similarities in symptomatology, particle size and mode of natural spread, indicate that the Indian isolates are best considered to be strains of PCV. However, no relationship was detected by immunosorbent electron microscopy either between the three Indian strains or between these and the West African strain. Although PCV has properties typical of the proposed furovirus group, no serological relationship was detected between any of the four strains and beet necrotic yellow vein, potato mop-top or soil-borne wheat mosaic viruses.

INTRODUCTION

Peanut clump virus (PCV) is a soil-borne virus (Thouvenel & Fauquet, 1981 *a*) first described from West Africa by Thouvenel *et al.* (1974). It has straight tubular particles of two predominant lengths, 190 and 245 nm, that contain two single-stranded RNA species of mol. wt. approx. 1.7×10^6 and 2.1×10^6 (Thouvenel & Fauquet, 1981 *b*). An apparently similar soil-borne virus was later obtained from stunted groundnut plants found in Punjab State, India; however, the Indian virus was serologically unrelated to the West African one and was therefore named Indian peanut clump virus (IPCV; Reddy *et al.*, 1983). More recently, isolates obtained from groundnut plants growing at two other localities in India, Bapatla and Hyderabad, were found to have little or no serological relationship either to the Punjab isolate or to one another (B. L. Nolt & D. V. R. Reddy, unpublished results). The work described in this paper was done to ascertain whether both RNA species of an Indian isolate are needed for infection, to determine whether, despite their apparent lack of antigenic relationship, affinities could be detected among the Indian and West African virus isolates, and to compare the properties of these viruses with those of other viruses with rod-shaped particles and soil-inhabiting plasmodiophoromycete fungus vectors.

METHODS

Sources and propagation of virus isolates. Three serologically distinct isolates of IPCV were used: IPCV-B from Bapatla, Andhra Pradesh, IPCV-H from Hyderabad, Andhra Pradesh and IPCV-L from Ludhiana, Punjab (B. L. Nolt & D. V. R. Reddy, unpublished results). Each isolate was cultured from an infected groundnut seed imported under licence from the Department of Agriculture and Fisheries for Scotland. These seeds were ground individually in 0.05 M-phosphate buffer pH 7.5, containing 0.2% thioglycerol and the extracts were inoculated at a series of dilutions to *Phaseolus vulgaris* cv. Topcrop and *Nicotiana clevelandii* plants. The IPCV-L isolate was

† Permanent address: International Crops Research Institute for the Semi-Arid Tropics, ICRISAT Patancheru P.O., Andhra Pradesh 502324, India.

passed five times consecutively through single lesions in *P. vulgaris*. Isolates IPCV-B and IPCV-H were each obtained from a single *N. clevelandii* plant infected by inoculum diluted almost to the endpoint of infectivity. All three isolates were cultured in mechanically inoculated *N. benthamiana* and *N. clevelandii* plants. Corundum-dusted leaves of *P. vulgaris* cv. Topcrop were used for local lesion infectivity assays. All plants were grown in an insect-free glasshouse at 20 to 25 °C with supplementary illumination from mercury vapour lamps.

A purified preparation of particles of West African PCV (PCV-WA) was provided by J. C. Thouvenel (Abidjan, Ivory Coast), of potato mop-top virus by P. R. Massalski (Scottish Crop Research Institute) and of a Japanese isolate of soil-borne wheat mosaic virus (SBWMV) by Y. Shirako (Sendai, Japan). Wheat leaves infected with a United States isolate of SBWMV were supplied by M. K. Brakke (Lincoln, Ne., U.S.A.), and *Chenopodium quinoa* leaves infected with a French isolate of beet necrotic yellow vein virus by C. Putz (Colmar, France). Materials from overseas were imported under licence from the Department of Agriculture and Fisheries for Scotland.

Purification of IPCV particles. Infected *N. clevelandii* leaves were homogenized in 0.05 M-phosphate buffer pH 8.0, containing 0.2% thioglycerol and 0.01 M-sodium diethyl dithiocarbamate (4 ml buffer/g leaf). The extract was filtered through two layers of cheesecloth, shaken with 1/9 vol. chloroform for 15 min and centrifuged in a Sorvall GSA rotor for 10 min at 5000 r.p.m. Virus particles were precipitated by adding 1.17 g NaCl and 4 g polyethylene glycol 6000 (PEG) to each 100 ml aqueous phase and keeping the mixture at 4 °C for 1.5 to 2 h. The precipitate was sedimented by centrifuging at 10000 r.p.m. for 10 min and was resuspended in 0.02 M-sodium borate, 0.03 M-potassium phosphate buffer, pH 8.3 containing 0.2 M-urea (BPU) and clarified at 5000 r.p.m. for 10 min. Each 25 ml supernatant fluid was layered over 12 ml 30% sucrose solution prepared in BPU containing 4% PEG and 0.2 M-NaCl and centrifuged in a Beckman SW27 rotor for 2 h at 24000 r.p.m. Pellets were resuspended in BPU and clarified by centrifuging at 5000 r.p.m. for 10 min. Each 8 ml supernatant fluid was floated on a gradient, prepared about 16 h previously by layering 6 ml each of 10%, 20% and 30% sucrose on 12 ml 40% sucrose, all in BPU. The gradients were then centrifuged in a SW27 rotor for 2 h at 24000 r.p.m. The light-scattering zone containing virus particles, located at 2.0 to 2.5 cm from the bottom of the tube, was collected using a hypodermic needle and the sucrose removed by a cycle of ultracentrifugation and resuspension of the pellets in 0.01 M-Tris-HCl, 0.06 M-NaCl, 0.003 M-EDTA, pH 8.6 (STE buffer). The final preparations had an A_{260}/A_{280} ratio of 1.2 (not corrected for light scattering). Yields of virus particles were up to 3 mg/100 g leaf, depending on the virus isolate and assuming $E_{1\text{cm},260}^{0.1\%} = 3.0$.

Preparation of virus RNA. SDS was added to purified virus particles in STE buffer to give a concentration of 0.5%. After 15 min at room temperature, the preparation was mixed for 3 min with an equal volume of phenol mixture (STE-saturated phenol containing 10% *m*-cresol and 0.1% 8-hydroxyquinoline). The mixture was kept at 60 °C for 2 min and then centrifuged for 20 min at 5000 r.p.m. The aqueous phase was removed, extracted with diethyl ether at least four times and the RNA precipitated from 70% ethanol at -20 °C.

Electrophoresis of glyoxalated IPCV RNA for mol. wt. determination. RNA, obtained as described above, was washed twice in 70% ethanol. IPCV RNA and marker RNA samples were denatured with 1 M-glyoxal and 50% (v/v) DMSO as described by Murant *et al.* (1981). Denatured RNA (8 to 10 µg/gel) was electrophoresed in cylindrical gels (85 × 6 mm) of 0.75% agarose in 0.01 M-sodium phosphate buffer pH 7.0, for about 2 h at 5 V/cm. Gels were stained with toluidine blue O as described by Murant *et al.* (1981). The markers were tobacco mosaic virus RNA (mol. wt. 2.21×10^6 for Na salt; Goelet *et al.*, 1982) and *Escherichia coli* ribosomal RNA (mol. wt. 1.01×10^6 and 0.53×10^6 for the Na salts; Brosius *et al.*, 1978, 1980; Carbon *et al.*, 1978).

Electrophoresis of IPCV RNA for infectivity assay. RNA precipitated in 70% ethanol was resuspended in TPE buffer (1 × TPE is 3.6 mM-Tris-HCl, 3 mM-NaH₂PO₄, 0.1 mM-EDTA, pH 7.8) containing 8 M-urea and 0.5% SDS. Samples of 8 to 12 µg RNA were electrophoresed in 10 × TPE for 4 h at 6 V/cm in cylindrical gels of 6 mm diameter, containing 2.1% acrylamide and 0.5% agarose. Immediately after electrophoresis, the gels were stained for 2 min with 0.05% toluidine blue O in 10 × TPE, then destained in the same buffer for about 5 min. This procedure resulted in peripheral staining of two RNA bands. Each band was cut out and a 2 mm gel slice between the two bands was discarded. Corresponding slices from five to six gels were pooled and disrupted in a tissue grinder with 0.5 ml 0.02 M-sodium phosphate buffer pH 8.0, containing 500 µg/ml magnesium bentonite, prepared as described by Dunn & Hitchborn (1965). After storage overnight at 4 °C, the extracts were clarified by centrifugation at 7000 r.p.m. for 20 min in a Sorvall SS34 rotor.

Treatment of virus RNA with proteinase K. IPCV-L RNA (30 µg/ml) was treated with proteinase K (200 µg/ml) at 30 °C for 4 h by the method of Harrison & Barker (1978). The RNA was then recovered from the samples after phenol extraction as described above. For infectivity assay, the material precipitated from 70% ethanol was resuspended in 0.02 M-sodium phosphate buffer pH 8.0, containing 500 µg/ml magnesium bentonite.

Infectivity assay. Plastic gloves were worn for manual inoculation of corundum-dusted leaves with samples containing virus particles or virus RNA. Each sample was inoculated to at least six half-leaves of *P. vulgaris* cv. Topcrop. Treatments were distributed among half-leaves according to a Latin square design and lesions were counted about 5 days after inoculation.

Immunosorbent electron microscopy (ISEM). Tests were as described by Roberts & Harrison (1979), with antiserum dilutions of 1/1000, and 2% ammonium molybdate, pH 7.0, as the negative strain. Numbers of particles per 1000 μm^2 of electron microscope grid were estimated by the method of Roberts (1980). Factors of increase in numbers of particles trapped were calculated relative to the numbers on grids coated with normal serum. Little importance is attached to increase factors of <3.0. The virus antisera used and their sources were as follows: barley stripe mosaic (D. A. Govier, Harpenden, U.K.), beet necrotic yellow vein (T. Tamada, Naganuma, Japan), hypochoeris mosaic (A. A. Brunt, Littlehampton, U.K.), IPCV-B, -H and -L isolates (ICRISAT), nicotiana velutina mosaic (this Institute), PCV-WA (J. C. Thouvenel, Abidjan, Ivory Coast), potato mop-top (this Institute) and SBWMV (M. K. Brakke, Lincoln, Ne., U.S.A.).

Preparation of complementary DNA and hybridization with RNA. ^3H -labelled DNA copies (cDNA) of unfractionated virus RNA were prepared by the method of Taylor *et al.* (1976), using the reaction conditions of Robinson *et al.* (1980). Mixtures of cDNA and unfractionated RNA from each virus strain, in 0.18 M-NaCl, 0.01 M-Tris-HCl pH 7, 1 mM-EDTA, 0.05% SDS, were incubated to a R_{0t} value of 0.85 mol. s/l. Hybrid formation was assayed using S1 nuclease as described by Robinson *et al.* (1980).

RESULTS

Size and infectivity of IPCV RNA species

When grown at Dundee, IPCV-B, IPCV-H and IPCV-L all produced systemic mosaic symptoms in *N. benthamiana*. In *N. clevelandii*, IPCV-L produced a severe mosaic whereas the other two isolates caused almost symptomless systemic infections. Countable local lesions were not produced in either species but symptom-bearing systemically infected leaves of each were satisfactory sources of virus particles for purification. However, necrotic local lesions were produced in *P. vulgaris* cv. Topcrop and chlorotic local lesions in *C. amaranticolor*. The lesions induced by IPCV-L in *P. vulgaris* were more discrete than those of the other two isolates, and were the most suitable for quantitative infectivity assay. IPCV-L RNA at 5 $\mu\text{g}/\text{ml}$ produced about 25 lesions per half-leaf of *P. vulgaris*; the extracted RNA was about 0.5% as infective as the same amount of RNA contained in virus particles.

Glyoxalated IPCV-L RNA produced two main bands on electrophoresis in agarose gels; the estimated mol. wt. of the two components were 1.83×10^6 and 1.35×10^6 , respectively. These values are lower than the estimates obtained in non-denaturing conditions (1.9×10^6 and 1.65×10^6 ; B. L. Nolt & D. V. R. Reddy, unpublished results) but the ratio of the two values obtained in denaturing conditions is almost the same as the ratio of the two modal lengths of IPCV-L particles in purified preparations (250 and 184 nm; Reddy *et al.*, 1983). It can safely be assumed that the 250 nm particles contain the larger RNA species, RNA-1, and that the 184 nm particles contain the smaller RNA species, RNA-2. RNA-2 occurred in slightly larger amounts than RNA-1.

When RNA-1 and RNA-2 of IPCV-L were separated by electrophoresis in non-denaturing conditions, and extracted separately from gel slices, the resulting samples produced few lesions in *P. vulgaris*. However, when the samples were mixed, the number of lesions was greatly enhanced (Table 1), suggesting that RNA-1 and RNA-2 are different parts of the IPCV genome and that both are needed for lesion formation in *P. vulgaris*. Treatment of IPCV-L RNA with proteinase K had little effect on infectivity, and there was no evidence for the existence of a genome-linked protein that plays an important role in infection.

Sequence homology between RNA preparations from IPCV strains and PCV

Preliminary experiments using RNA from IPCV-L and the homologous cDNA preparation showed that hybridization was essentially complete at R_{0t} values greater than 0.2 mol. s/l. Table 2 shows the results of experiments in which RNA preparations from the three IPCV strains and their cDNA copies were hybridized in all possible combinations. For each cDNA preparation the apparent percentage sequence homology observed depends on the relative proportions of copies of each RNA species in the preparation. Thus, although homology values obtained with each cDNA preparation and different RNA preparations are directly comparable, those obtained with different cDNA preparations are not. All three IPCV strains clearly have substantial common sequences, with IPCV-H and IPCV-B seeming to be more closely related

Table 1. *Infectivity of RNA species of IPCV*

	Infectivity* of			Increase factor†
	RNA-1	RNA-2	RNA-1 + RNA-2	
Expt. 1	2	0	16	8.0
Expt. 2	7	3	119	11.9

* Figures are total numbers of lesions in six or eight half-leaves of French bean. Lesions produced by all inocula were of the same type.

† (Number of lesions produced by RNA-1 + RNA-2 mixed) ÷ (sum of numbers of lesions produced by RNA-1 and RNA-2 separately).

Table 2. *Apparent percentage sequence homology between RNA preparations from IPCV and PCV*

RNA of	cDNA to unfractionated RNA of			
	IPCV-B	IPCV-H	IPCV-L	PCV-WA
IPCV-B	100	86*	63	23
IPCV-H	75	100	64	24
IPCV-L	51	67	100	27
PCV-WA	29	41	35	100

* Apparent % sequence homology, calculated according to Gonda & Symons (1978), in tests using unfractionated RNA preparations hybridized to a R_0 t value of 0.85 mol. s/l. Each value is the mean of at least two determinations.

Table 3. *Relationships among viruses with possible affinities to IPCV, assessed by ISEM*

Antiserum*	Virus*						
	IPCV-B	IPCV-H	IPCV-L	SBWMV-J	SBWMV-US	PMTV	BNYVV-F
IPCV-B	6.4†	<2	<2	<2	-	<2	-
IPCV-H	<2	5.5	<2	<2	-	<2	-
IPCV-L	<2	<2	11.5	<2	<2	<2	<2
PCV-WA	<2	-	<2	<2	<2	<2	<2
SBWMV-US	<2	<2	<2	23.5	57.1	13.6	<2
PMTV	<2	<2	<2	<2	2.8	30.3	<2
BNYVV-J	<2	-	<2	<2	<2	<2	13.1
BSMV	<2	-	<2	-	-	<2	-
HyMV	<2	-	<2	-	<2	<2	<2
NVMV	<2	-	<2	-	-	<2	<2

* Abbreviations are as in Methods, and: BNYVV-F and -J, French and Japanese isolates of beet necrotic yellow vein virus; BSMV, barley stripe mosaic virus; HyMV, hypochoeris mosaic virus; NVMV, nicotiana velutina mosaic virus; PMTV, potato mop-top virus; SBWMV-J and -US, Japanese and United States isolates of soil-borne wheat mosaic virus.

† Figures are mean factors of increase in particle numbers on antiserum-coated grids as compared with normal serum-coated grids. Homologous reactions are in bold type.

than either is to IPCV-L. In addition, each strain of IPCV shares sequences with PCV-WA but these homologies are less extensive than those between the IPCV strains (Table 2). In further tests, no sequence homology was detected between IPCV-L and pepper ringspot tobnavirus.

Serological relationships of isolates

Table 3 summarizes a series of tests made by ISEM to assess the extent of the serological relationship between the three Indian and one West African isolates, and between these and some other viruses with particles of similar shape. Among the viruses known or thought likely to have plasmodiophoromycete fungus vectors, the only relationship detected was that between

potato mop-top virus and SBWMV (Randles *et al.*, 1976). Beet necrotic yellow vein virus, PCV-WA and each of the IPCV isolates seemed unrelated to any other virus tested. However, the tests detected relationships between Japanese and United States isolates of SBWMV and between Japanese and French isolates of beet necrotic yellow vein virus.

DISCUSSION

The results of the nucleic acid hybridization tests show that the three serologically unrelated IPCV isolates have extensive nucleotide sequence homologies not only with one another but also with PCV-WA. IPCV and PCV also have particles of the same shape and lengths, cause similar diseases in groundnut, have similar experimental host ranges including *Chenopodium* and *Nicotiana* spp., and both are soil-borne and seed-transmitted (Thouvenel & Fauquet, 1981*b*; Reddy *et al.*, 1983). But for their apparent lack of serological relationship, one would have no hesitation in classing the three IPCV isolates as strains of PCV. This situation has a precedent in the tobnaviruses. For example, strains of tobacco rattle virus (TRV) have much nucleotide sequence homology and can be shown to produce pseudo-recombinants by reassortment of their two genome parts although their particles may have little or no detectable antigenic relationship (Robinson & Harrison, 1985). This reflects the great genetic variation found in TRV RNA-2, which contains the particle protein gene. Although no information is available on the compatibility of the genome parts of IPCV with those of PCV, it seems very probable that the pattern of genome variation in PCV resembles that found in TRV and we therefore propose that the IPCV isolates should be considered to be strains of PCV.

PCV has a combination of properties that distinguish it from all well characterized groups of plant viruses. Among viruses with rod-shaped particles, PCV resembles tobnaviruses in having a bipartite ssRNA genome that is apparently not polyadenylated, with an RNA-2 that contains the particle protein gene and an RNA-1 that is translated *in vitro* to give two large polypeptides coded by overlapping sequences (Mayo & Reddy, 1985). However, tobnaviruses differ from PCV in that their RNA-1 can cause local and systemic symptoms in plants on its own and they have nematode, not fungal, vectors (Harrison & Robinson, 1978).

Among other viruses with rod-shaped particles, PCV has its strongest affinities with SBWMV. The latter has a *Polymyxa* fungal vector (Rao & Brakke, 1969), as probably also does PCV (Thouvenel & Fauquet, 1981*a*), and isolates of SBWMV typically produce particles of two predominant lengths (Brandes *et al.*, 1964) containing two ssRNA species that are not polyadenylated and that both seem to be needed for systemic infection of wheat (Shirako & Brakke, 1984; Hsu & Brakke, 1985). Moreover, translation *in vitro* of SBWMV RNA-1 gives a large polypeptide and that of RNA-2 gives the virus particle protein (Hsu & Brakke, 1983). Finally, PCV in India (D. V. R. Reddy, unpublished results), like SBWMV in North America, infects wheat crops. The two viruses differ both in the lengths of their particles, which typically are about 300 and 160 nm for SBWMV (Brandes *et al.*, 1964) compared with about 245 and 190 nm for PCV, and in the relative sizes of their two genome segments. Shirako & Brakke (1984), who recognized the differences between SBWMV and members of well characterized virus groups such as the tobamoviruses, suggested that SBWMV should become the type member of a new group of fungus-transmitted viruses with rod-shaped particles and tentatively named it the furovirus group. PCV is probably the strongest candidate among other plant viruses for membership of such a group. Other possible members are potato mop-top and beet necrotic yellow vein viruses, but not tobacco stunt virus, which has a chytrid fungus vector and particles that yield dsRNA (Kuwata & Kubo, 1984).

We thank M. K. Brakke, A. A. Brunt, D. A. Govier, P. R. Massalski, C. Putz, Y. Shirako, T. Tamada and J. C. Thouvenel for generously providing virus and/or antiserum samples and Moira Brunton and Ann Jenkins for skilled technical assistance. This paper is submitted as Journal Article no. 526 by the ICRISAT.

REFERENCES

- BRANDES, J., PHILLIPPE, M. R. & THORNBERRY, H. H. (1964). Electron microscopy of particles associated with soil-borne wheat mosaic. *Phytopathologische Zeitschrift* **50**, 181-190.

- BROSIOUS, J., PALMER, M. L., KENNEDY, P. J. & NOLLER, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proceedings of the National Academy of Sciences, U.S.A.* **75**, 4801-4805.
- BROSIOUS, J., DULL, T. J. & NOLLER, H. F. (1980). Complete nucleotide sequence of a 23S ribosomal RNA gene from *Escherichia coli*. *Proceedings of the National Academy of Sciences, U.S.A.* **77**, 201-204.
- CARBON, P., EHRESMANN, C., EHRESMANN, B. & EBEL, J. P. (1978). The sequence of *Escherichia coli* ribosomal 16S RNA determined by new rapid gel methods. *FEBS Letters* **94**, 152-156.
- DUNN, D. B. & HITCHBORN, J. H. (1965). The use of bentonite in the purification of plant viruses. *Virology* **25**, 171-192.
- GOELET, P., LOMONOSSOFF, G. P., BUTLER, P. J. G., AKAM, M. E., GAIT, M. J. & KARN, J. (1982). Nucleotide sequence of tobacco mosaic virus RNA. *Proceedings of the National Academy of Sciences, U.S.A.* **79**, 5818-5822.
- GONDA, T. J. & SYMONS, R. H. (1978). The use of hybridization analysis with complementary DNA to determine the RNA sequence homology between strains of plant viruses: its application to several strains of cucumoviruses. *Virology* **88**, 361-370.
- HARRISON, B. D. & BARKER, H. (1978). Protease-sensitive structure needed for infectivity of nepovirus RNA. *Journal of General Virology* **40**, 711-715.
- HARRISON, B. D. & ROBINSON, D. J. (1978). The tobnaviruses. *Advances in Virus Research* **23**, 25-77.
- HSU, Y. H. & BRAKKE, M. K. (1983). Translation of soil-borne wheat mosaic virus RNAs in rabbit reticulocyte lysate. *Phytopathology* **73**, 790.
- HSU, Y. H. & BRAKKE, M. K. (1985). Sequence relationships among soil-borne wheat mosaic virus RNA species and terminal structures of RNA II. *Journal of General Virology* **66**, 915-919.
- KUWATA, S. & KUBO, S. (1984). Properties of two *Ospidium*-transmitted viruses: tobacco stunt and lettuce big vein. *Sixth International Congress of Virology, Sendai, Japan, 1984*. Abstract P45-2.
- MAYO, M. A. & REDDY, D. V. R. (1985). Translation products of RNA from Indian peanut clump virus. *Journal of General Virology* **66**, 1347-1351.
- MURANT, A. F., TAYLOR, M., DUNCAN, G. H. & RASCHKÉ, J. H. (1981). Improved estimates of molecular weight of plant virus RNA by agarose gel electrophoresis and electron microscopy after denaturation with glyoxal. *Journal of General Virology* **53**, 321-332.
- RANDLES, J. W., HARRISON, B. D. & ROBERTS, I. M. (1976). *Nicotiana velutina* mosaic virus: purification, properties and affinities with other rod-shaped viruses. *Annals of Applied Biology* **84**, 193-204.
- RAO, A. S. & BRAKKE, M. K. (1969). Relation of soil-borne wheat mosaic virus and its fungal vector, *Polymyxa graminis*. *Phytopathology* **59**, 581-587.
- REDDY, D. V. R., RAJESHWARI, R., HIZUKA, W., LESEMANN, D. E., NOLT, B. L. & GOTO, T. (1983). The occurrence of Indian peanut clump, a soil-borne virus disease of groundnuts (*Arachis hypogaea*) in India. *Annals of Applied Biology* **102**, 305-310.
- ROBERTS, I. M. (1980). A method for providing comparative counts of small particles in electron microscopy. *Journal of Microscopy* **118**, 241-245.
- ROBERTS, I. M. & HARRISON, B. D. (1979). Detection of potato leafroll and potato mop-top viruses by immunosorbent electron microscopy. *Annals of Applied Biology* **93**, 289-297.
- ROBINSON, D. J. & HARRISON, B. D. (1985). Unequal variation in the two genome parts of tobnaviruses and evidence for the existence of three separate viruses. *Journal of General Virology* **66**, 171-176.
- ROBINSON, D. J., BARKER, H., HARRISON, B. D. & MAYO, M. A. (1980). Replication of RNA-1 of tomato black ring virus independently of RNA-2. *Journal of General Virology* **51**, 317-326.
- SHIRAKO, Y. & BRAKKE, M. K. (1984). Two purified RNAs of soil-borne wheat mosaic virus are needed for infection. *Journal of General Virology* **65**, 119-127.
- TAYLOR, J. M., ILLMENSEE, R. & SUMMERS, J. (1976). Efficient transcription of RNA into DNA by avian sarcoma virus polymerase. *Biochimica et biophysica acta* **442**, 324-330.
- THOUVENEL, J. C. & FAUQUET, C. (1981a). Further properties of peanut clump virus and studies on its natural transmission. *Annals of Applied Biology* **97**, 99-107.
- THOUVENEL, J. C. & FAUQUET, C. (1981b). Peanut clump virus. *Commonwealth Mycological Institute/Association of Applied Biologists Descriptions of Plant Viruses*, no. 235.
- THOUVENEL, J. C., GERMANI, G. & PFEIFFER, P. (1974). Preuve de l'origine virale de rabougrissement ou "clump" de l'arachide en Haute-Volta et au Sénégal. *Comptes rendus hebdomadaires des séances de l'Académie des sciences, série D* **278**, 2847-2849.

(Received 14 May 1985)