Ann. appl. Biol. (1993), **122**, 467–479
*Printed in Great Britain

Chickpea chlorotic dwarf virus, a new leafhopper-transmitted geminivirus of chickpea in India[1]

By N. M. HORN, S. V. REDDY, I. M. ROBERTS* and D. V. R. REDDY

Virology Unit, Legumes Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India

**Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK*

(Accepted 24 November 1992)

Summary

A disease of chickpea in India, characterised by chlorosis, severe stunting and phloem browning, was shown to be caused by a geminivirus. This virus was transmitted by the leafhopper *Orosius orientalis* from chickpea to chickpea and several other plant species. A method for purification of this virus was devised and a polyclonal antiserum produced. The majority of the purified particles were geminate. The size of the coat protein was shown to be 32 kD and the nucleic acid was shown to be circular ssDNA of 2900 nucleotides. By immunosorbent electron microscopy this virus was shown to be unrelated to the leafhopper-transmitted geminiviruses known to infect dicotyledons such as beet curly top, bean summer death and tobacco yellow dwarf viruses. On the basis of particle morphology, leafhopper transmission, host range and serology this virus was considered to be a new, hitherto undescribed, geminivirus and was named chickpea chlorotic dwarf virus.

Key words: Geminivirus, chickpea stunt, chickpea chlorotic dwarf virus, leafhopper, India, *Orosius orientalis*, bean summer death virus, tobacco yellow dwarf virus

Introduction

Stunt is an important viral disease of chickpea (*Cicer arietinum*) in many chickpea-growing countries. The characteristic symptoms are internode shortening, plant stunting or dwarfing, leaf reddening in the case of desi-type and yellowing in the case of kabuli-type chickpeas and phloem browning in the collar region (Nene *et al.*, 1991; Nene & Reddy, 1987; Duffus, 1979). Bean leafroll luteovirus (BLRV) has been mentioned to be associated with the disease in India (Reddy, Nene & Verma, 1979; Nene *et al.*, 1991). Several other luteoviruses have been found to infect chickpea, *viz.* pea leafroll virus (= BLRV) in Iran (Kaiser & Danesh, 1971), subterranean clover red leaf (SCRLV), beet western yellows (BWYV) and legume yellows (LYV) viruses in California (Bosque-Perez & Buddenhagen, 1990; Duffus, 1979).

Several chickpea plants showing stunt symptoms were collected from different parts of India and processed, several plants together, for the isolation and purification of luteoviruses. Although luteoviruses were recovered from these field-infected plants, samples from several places were also found to contain a geminivirus, so far unknown for this

[1]Submitted as Journal Article No. 1390 by ICRISAT

plant species. It was therefore further investigated to know if it is different from other geminiviruses described so far. Since the vector of this virus was not known, the initial study of this virus was difficult. Only after the discovery of its leafhopper vector could the virus be transmitted artificially, isolated and characterised and was found to differ from other leafhopper-transmitted geminiviruses. This paper reports the host range, transmission, and physico-chemical properties of this geminivirus.

Materials and Methods

Virus isolate

The virus isolate studied in detail was obtained from field-infected chickpea plants (cv. WR 315) collected at Hisar, Haryana State (India), showing reddening of the leaflets, phloem browning and plant stunting.

Mechanical inoculation

Young chickpea leaflets showing typical symptoms were triturated in 50 mM potassium phosphate buffer (pH 7.0) containing 750 µl/litre thioglycerol (1 g tissue/9 ml buffer). The extract was used to inoculate manually Carborundum-dusted leaves of chickpea, pea and tobacco plants.

The insects

A culture of the leafhopper *Orosius orientalis* Matsumura, previously named *O. albicinctus* Distant, was maintained on *Sesamum indicum* and *Crotalaria juncea* and a culture of the whitefly *Bemisia tabaci* was maintained on cotton (*Gossypium hirsutum*).

A partially purified virus preparation containing sucrose (150 g/litre) was used to feed *O. orientalis* and *B. tabaci* adults through a parafilm® membrane. After a one-day acquisition-access period, the leafhoppers and whiteflies were allowed a two-day inoculation-access period on pea (*Pisum sativum* cv. Bonneville) and chickpea (cv. WR 315) plants. The virus isolated from chickpea plants was maintained on pea plants (cv. Bonneville). For this purpose *O. orientalis* was allowed a two-day acquisition-access period on infected pea plants followed by a two-day inoculation-access period on healthy pea plants. Back inoculation from pea to chickpea reproduced the characteristic symptoms in chickpea.

Host-range studies

At least six plants of each species were grown in a glasshouse with temperatures ranging from 25–30°C. Young adults of *O. orientalis*, given access to infected pea plants for 2 days, were used. Inoculation feeding periods on test plants were 2 days using 25 leafhoppers per pot containing 2–3 test plants. The test plants were observed visually for symptoms and tested by double antibody sandwich (DAS)-ELISA and immunosorbent electron microscopy (ISEM) for the presence of the virus.

Virus purification

The procedure described by Van den Heuvel, de Blank, Goldbach & Peters (1990) for potato leafroll virus was used with slight modifications. Pea plants showing stunting, leaf-rolling and chlorosis were harvested 10–15 days after inoculation with infective leafhoppers and were stored at −70°C. Normally 100 g of frozen tissue was processed each time. All steps were carried out at room temperature whereas the centrifugations were done at 15°C.

Tissue was homogenised in a blender with two volumes of 100 mM sodium citrate buffer, pH 6.0 (SCB), containing ethanol (5 ml/litre), thioglycollic acid (1 ml/litre) and Celluclast® (30 ml/litre). The extract was stirred for 3 h, whereafter chloroform (250 ml/litre) and butanol (250 ml/litre) were added. The mixture was stirred for 5 min and clarified by centrifugation (13 680 ***g***, 15 min). Triton X-100 was added (1.0 ml/litre) to the aqueous phase and the mixture stirred for 30 min, then polyethylene glycol (mol. wt 8000) at 80 g/litre and sodium chloride at 23.6 g/litre were added and the mixture was stirred for 20 min before keeping at room temperature for 2 h. The resulting precipitate was collected by centrifugation (13 680 ***g***, 20 min) and resuspended in 30 ml of 100 mM SCB, containing ethanol (50 ml/litre). This mixture was stirred for 30 min, incubated at room temperature for 16 h and clarified by centrifugation (7100 ***g***, 15 min). The supernatant was loaded on to 15 ml of 30% sucrose, prepared in SCB in Beckman R45 rotor tubes and centrifuged at 185 500 ***g*** for 4 h. Each pellet was resuspended in 1 ml SCB, loaded onto a 100–400 g/litre sucrose gradient (in a Beckman SW 40 rotor) and centrifuged for 3 h at 110 000 ***g***. The gradients were prepared by layering 2.7 ml of each of 100, 200, 300 and 400 g/litre sucrose and stored overnight at 4°C. Fractions of the gradient were collected with a bent needle attached to a syringe, diluted in SCB, and centrifuged in a Beckman R50 rotor at 150 000 ***g*** for 4 h. Pellets were resuspended in 100 μl of 10 mM Tris-HCl buffer, pH 8, containing 1 mM EDTA.

Electron microscopy

Three-hundred mesh copper grids coated with a carbon film were inverted onto 10-μl drops of purified virus suspension for 10 min, stained with 1% uranyl acetate and examined with a Philips 201 C electron microscope.

Antiserum production

Purified virus (20–80 μg) was emulsified with an equal volume of Freund's incomplete adjuvant and injected intramuscularly into an inbred New Zealand White rabbit at weekly intervals. After four injections the rabbit was bled at weekly intervals.

Immunosorbent electron microscopy (ISEM)

The method used was essentially that of Roberts & Harrison (1979). Grids were inverted on a 10-μl drop of antiserum (1:1000 dilution in 70 mM phosphate buffer, pH 6.5) for 1 h at 37°C followed by 10 min washing with the same buffer. The grids were inverted onto a 10-μl drop of partially purified virus suspension for 1 h, washed with distilled water, and stained with 2% ammonium molybdate, pH 6.5. Virus particles were counted in fifty viewing fields of a JEOL 100S electron microscope at 30 000× magnification, and the number of dimers per 1000 μm^2 was calculated (Roberts, 1980).

Enzyme-linked immunosorbent assay (ELISA)

Double antibody sandwich (DAS)-ELISA, as described by Clark & Adams (1977), and direct antigen coating (DAC)-ELISA, as described by Hobbs, Reddy, Rajeshwari & Reddy (1987), were used. Gammaglobulins were extracted with sodium sulphate (Hobbs *et al.*, 1987) and conjugated to alkaline phosphatase as described by Clark & Adams (1977), p-nitrophenyl phosphate was used at 1 mg/ml as substrate. In DAC-ELISA penicillinase was used as enzyme. Sodium penicillin (0.5 mg/ml) prepared in a bromothymolblue solution (0.15 g/litre) was then used as substrate (Sudarshana & Reddy, 1989). Various antisera used and their sources are given in Table 2.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out in a discontinuous slab gel for 16 h at 35 V (Laemmli, 1970). Samples were prepared in 62.5 mM Tris, pH 6.8, containing SDS (20 g/litre), mercaptoethanol (50 ml/litre) and glycerol (100 ml/litre), and then boiled for 5 min prior to loading onto the gels. Protein markers (Biorad) were phosphorylase b (97.4 kD), bovine serum albumin (66.2 kD), ovalbumin (42.6 kD), carbonic anhydrase (31 kD) and soybean trypsin inhibitor (21.5 kD). The gel was stained with silver nitrate as described by Reddy *et al.* (1990).

Nucleic acid extraction and gel analysis

Purified virus was treated with 0.5% SDS and protease K (5 μg/100 pg of virus) in 25 mM EDTA for 1 h at 55°C. The nucleic acid was extracted twice with a mixture of chloroform, phenol, isoamyl alcohol (24:24:1) and once with chloroform and isoamyl alcohol (48:1), and precipitated with ethanol and 2 M ammonium acetate at −20°C. Samples were resuspended in 10 mM Tris buffer containing 1 mM EDTA, pH 8.0, and were run for 1 h at 100 V at room temperature in a 1% agarose gel in 40 mM Tris acetate buffer containing 1 mM EDTA. Gels were stained for 5 min in ethidium bromide (1 μg/ml) and observed on a transilluminator. For treatment with RNase A (at 10 μg/ml), the nucleic acid was resuspended in 10 mM Tris-HCl, pH 7.8, containing 1 mM EDTA. For DNase I treatment (100 μg/ml) samples were resuspended in 10 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, and for treatment with nuclease S1 (1 unit per 100 ng nucleic acid) in 30 mM sodium acetate, 50 mM NaCl, 100 mM $ZnSO_4$ and 5% glycerol, pH 4.6. The reactions were stopped by adding EDTA to a final concentration of 10 mM and samples were analysed in an agarose gel as described above. Circular ssDNA molecular weight markers were derived from pUC 119 clones containing inserts of different sizes. Bacteriophage M13 KO7 was used as helper phage for production of the markers (Vieira & Messing, 1987). Samples for electron microscopy were prepared and spread as described by Murant, Taylor, Duncan & Raschke (1981).

Results

Transmission

Mechanical inoculation from chickpea, pea and tobacco (*Nicotiana tabacum* cv. White Burley) to chickpea, pea and tobacco was not successful; all possible combinations were tested. The virus could not be transmitted by *B. tabaci* but could be transmitted from chickpea to chickpea and to other plant species by *O. orientalis*. The leafhopper is also known to transmit sesamum phyllody (Vasudeva & Sahambi, 1955) and potato purple top roll (Singh, Nagaich & Bhargawa, 1983), both mycoplasma diseases.

Host-range studies

The host range of the virus using *O. orientalis* for transmission is presented in Table 1. The following plant species were not infected by the virus: *Cucumis sativus, Cajanus cajan, Arachis hypogaea, Medicago sativa, Vigna unguiculata, V. radiata* var. *radiata, V. mungo, Glycine max, Nicotiana rustica, N. occidentalis* and *Solanum melongena*. The characteristic symptoms observed on chickpea in the field, yellowing, reddening, plant stunting and phloem browning, could be reproduced on chickpea (Fig. 1 shows the stunting). A number of hosts, including *Nicotiana tabacum* (cvs White Burley and Samsum NN) and *Datura stramonium*, showed leaf rolling, yellowing and stunting. Plant species and cultivars which

Table 1. *Host range of the chickpea geminivirus as determined by inoculation using* O. orientalis; *symptoms observed and virus concentration as estimated by DAS-ELISA and ISEM (H = high, M = medium, L = low)*

Family species and cultivar	Symptoms	Virus concentration
Chenopodiaceae		
Beta vulgaris (sugarbeet)	chlorosis	H
Leguminosae		
Cicer arietinum (chickpea)	stunting, leafrolling reddening, yellowing phloem browning small leaves	M
Lens esculenta (lentil)	chlorosis, severe stunting	H
Phaseolus vulgaris		
cv. Top crop	stunting, leaf dropping, rapid death	H
cv. Burpy	none	M
Pisum sativum	stunt, leafrolling	H
cv. Bonneville (pea)	chlorosis	
Vica faba cv. Compakta (faba bean)	none	L
Solanaceae		
Datura stramonium	chlorosis, leafrolling, severe stunting	H
Lycopersicon esculentum (tomato)	none	M
Nicotiana benthamiana	stunting, chlorosis	H
Nicotiana glutinosa	stunting, vein chlorosis	H
Nicotiana tabacum		
cv. White Burley	stunting, leafrolling	H
cv. Samsum NN	small leaves, chlorosis	H

showed severe symptoms contained larger amounts of virus, as determined in ELISA and ISEM tests, than those which did not show strong symptoms. Two weeks after inoculation feeding, nymphs of *O. orientalis* appeared on faba bean, *Vicia faba* and lentil, *Lens esculenta*, indicating that the leafhopper deposited eggs on these plants. On none of the other plants tested nymphs appeared although the leafhopper survived well on all of them, including the plant species that were not infected by the virus.

Purification

The virus did not sufficiently scatter light to visualise the zones following centrifugation in a sucrose gradient. Therefore 1.5 ml fractions were drawn and pellets from each fraction were observed with an electron microscope. The absorption spectrum (200–300 nm) was recorded for samples containing virus particles. Fraction 2 (drawn at a depth of 30–40 mm from the top of the tube) contained the highest virus concentration (Fig. 2). Fraction 3, drawn at 40–50 mm from the top of the tube, contained mainly dimers and some trimers and fraction 4 (drawn 50–60 mm from the top of the tube) contained relatively more tri- and tetramers than fraction 2 and 3 (Fig. 3). Other fractions contained negligible amounts of virus particles. Virus yields of 0.5–0.6 mg/kg tissue were obtained from pea tissue assuming an extinction coefficient of 7.7 (Goodman & Bird, 1978). The A_{260}/A_{280} ratio was 1.4 and the dimers were 25 nm × 15 nm in size.

Fig. 1. The symptoms caused by the virus in chickpea after inoculation by *Orosius orientalis*, especially the stunting is clear. Left healthy, right inoculated plant, 2 weeks after inoculation.

Immunosorbent electron microscopy (ISEM)

The grids coated with the homologous antiserum could trap up to 2840 times as many particles as non-treated grids (Table 2). Except for the homologous antiserum, none of the antisera tested in ISEM gave a considerable increase in the number of particles trapped. Only bean summer death virus (BSDV) and tobacco yellow dwarf virus (TYDV) antisera gave a slight increase (2–8 times) over the control. This suggests a distant serological relationship with TYDV and BSDV.

ELISA

In DAS-ELISA, using tissue from *Phaseolus vulgaris* cv. Top Crop, the virus did not react with African cassava mosaic virus (ACMV) or Indian cassava mosaic virus (ICMV) polyclonal antisera nor with 27 monoclonal antibodies produced against ACMV and ICMV. In DAC-ELISA, using the chickpea virus purified from pea, the virus reacted strongly with antisera to BSDV and weakly with antisera to beet curly top virus (BCTV) and TYDV (Table 3).

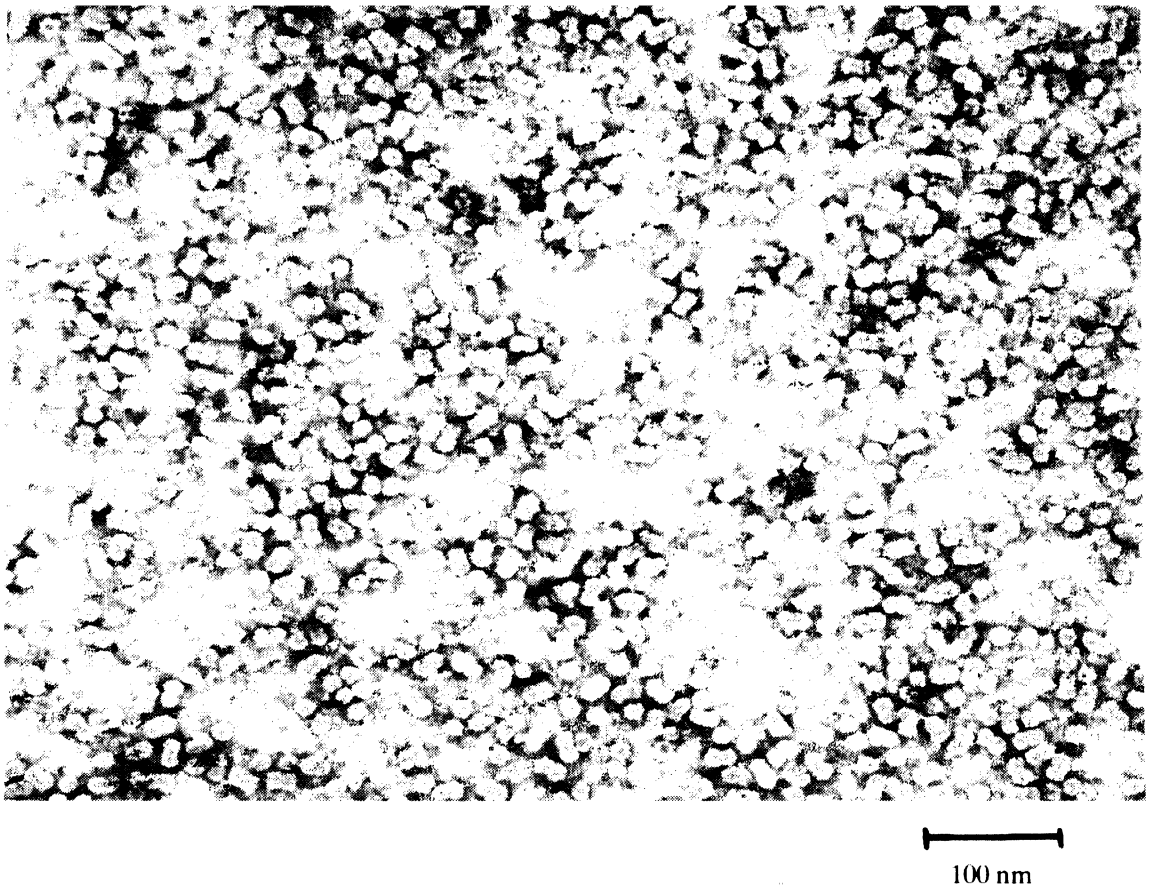

Fig. 2. Electronmicrograph of the virus particles present in fraction 2 of the sucrose gradient after purification from pea.

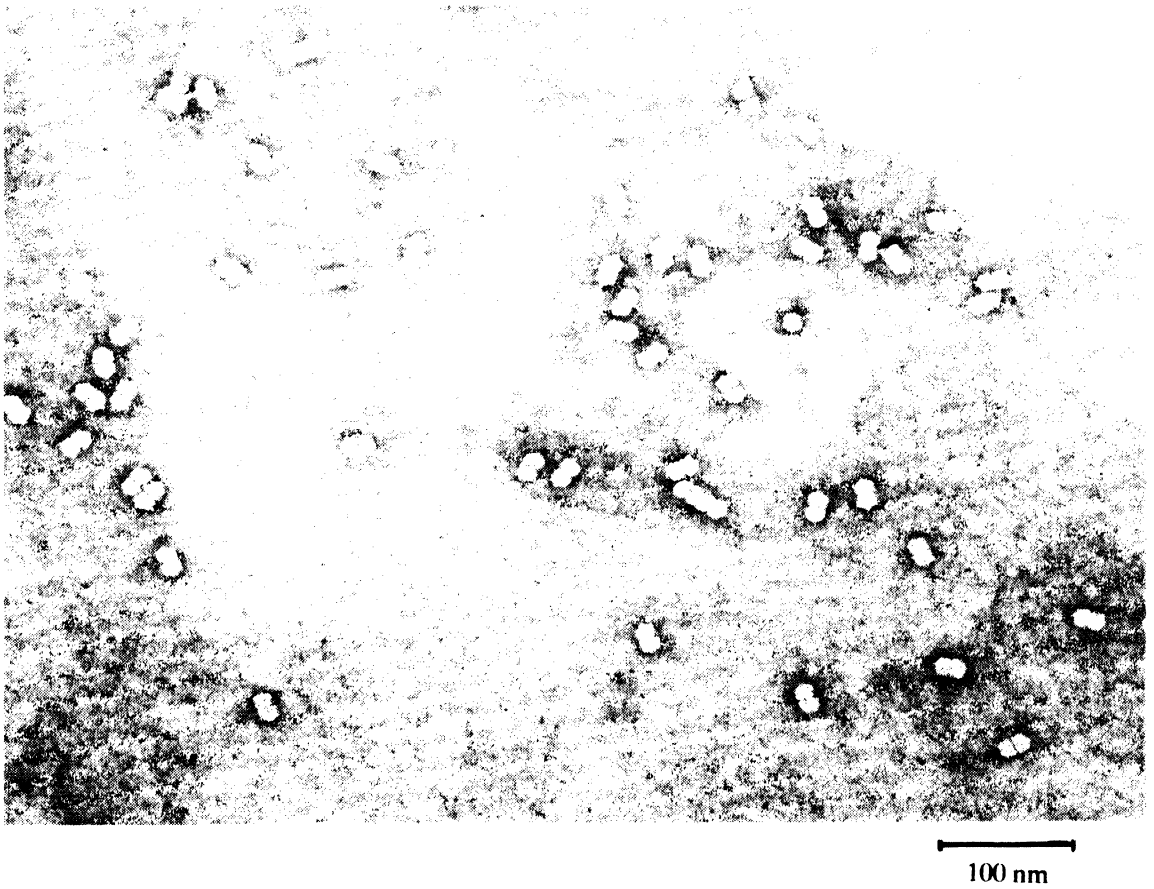

Fig. 3. Electronmicrograph of the virus particles present in fraction 4 of the sucrose gradient after purification from pea. Note the presence of dimer, trimer and tetramer particles.

Table 2. *Trapping of the chickpea geminivirus particles in immunosorbent electron microscopy by homologous and heterologous antisera ([a] = whitefly-transmitted geminivirus, [b] = leafhopper-transmitted geminivirus, [c] = nepovirus), as found in three separate experiments. The increase factor shows the increase in particle numbers on antiserum-coated grids as compared with the uncoated control. Sources of antisera: African cassava mosaic virus, B. D. Harrison; beet curly top virus, D. L. Mumford; chloris striate mosaic virus, R. I. B. Francki; maize streak virus, K. R. Bock; raspberry ringspot virus, SCRI; squash leaf curl virus, J. E. Duffus; tobacco yellow dwarf virus, J. E. Thomas; bean summer death virus, J. E. Thomas; and wheat dwarf virus, K. Lindsten*

	Number of dimers per 1000 μm²			
Virus antiserum	Expt 1	Expt 2	Expt 3	Increase factor
Homologous	21336	34111	6090	130–2840
African cassava mosaic[a]	13	—	—	0.37
Beet curly top[b]	27	—	—	0.77
Chloris striate mosaic[b]	—	0	—	—
Maize streak[b]	—	13	—	1.08
Raspberry ringspot[c]	0	37	—	—
Squash leaf curl[a]	0	—	—	—
Tobacco yellow dwarf[b]	289	51	92	2–8
Bean summer death[b]	—	—	118	2.5
Wheat dwarf[b]	—	0	—	—
Control (= no serum)	35	12	48	—

SDS-PAGE

A single protein band of 32 kD (average of four determinations) was detected from purified virus preparations (Fig. 4).

Nucleic acid characterisation

A single nucleic acid band of 2900 nucleotides (average of four determinations) was observed from purified virus samples (Fig. 5). It was digested by DNase I and nuclease S1 but not by RNase A, (Fig. 5). Circular nucleic-acid strands were observed by electron microscopy (Fig. 6).

Discussion

On the basis of the structure of its particles, the virus was recognised as a geminivirus. The presence of circular, single-stranded DNA further corroborated this. Only after many efforts, using different insect species, the vector of the virus was found to be the leafhopper *Orosius orientalis*. The virus possesses a single coat protein subunit with a molecular weight of 32 000 daltons and circular ssDNA with a molecular size of 2900 bases, both within the range of the geminiviruses (Harrison, 1985).

Serological relationships were tested with a range of geminivirus antisera. It is serologically unrelated to ACMV and SLCV. These two viruses were included in the test because the majority of the whitefly-transmitted geminiviruses are serologically related to them (Roberts, Robinson & Harrison, 1984). The chickpea virus is also unrelated to MSV, CSMV and WDV, the three leafhopper-transmitted geminiviruses infecting monocotyledonous plant species. In ISEM no relation was found with BCTV but a possibly distant relationship was found with BSDV and TYDV. In DAC-ELISA, however, the chickpea virus reacted strongly with BSDV antiserum and weakly with BCTV and TYDV antisera. The difference found in the serological reactions of the virus in ISEM and DAC-ELISA might be explained

Table 3. *The serological relationship of the chickpea geminivirus with BCTV, BSDV and TYDV as tested by DAC-ELISA using chickpea geminivirus purified from pea, homologous antiserum (1/1000), three heterologous antisera (BCTV, BSDV, TYDV, all 1/500) and penicillinase as enzyme. Absorption at 620 nm subtracted from the values for comparable healthy plants. Readings were taken 90 min after addition of substrate. The figures given are means of three replicates*

Amount Virus/Well	Homologous antiserum	Antisera BCTV	BSDV	TYDV
1 µg	1.830	0.658	1.480	0.479
100 ng	0.752	0.010	0.770	0.033
10 ng	0.106	0.021	0.029	0.025

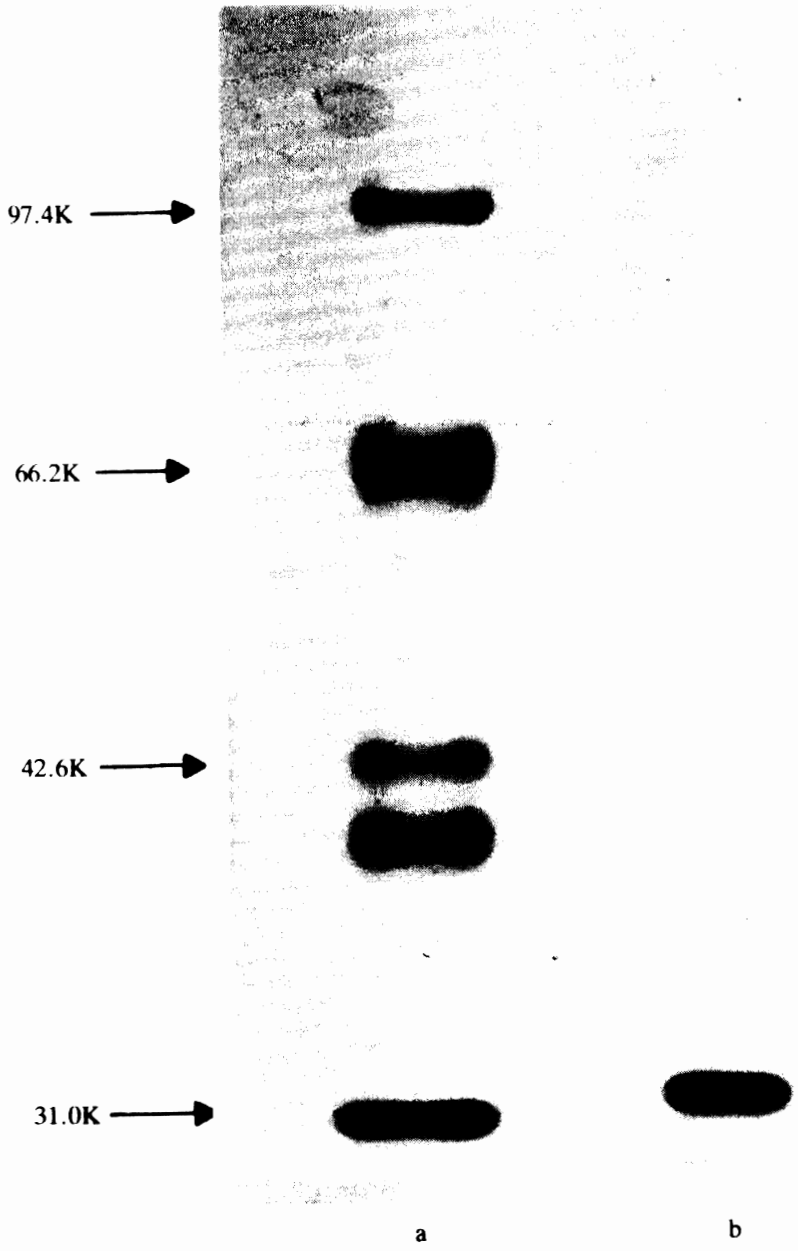

Fig. 4. The results of SDS-PAGE of (a) protein markers (97.4; 66.2; 42.6 and 31 kD) and (b) the virus coat protein. The 42.6 kD protein forms two bands, the lower one being a degradation product. The coat protein is approximately 32 kD.

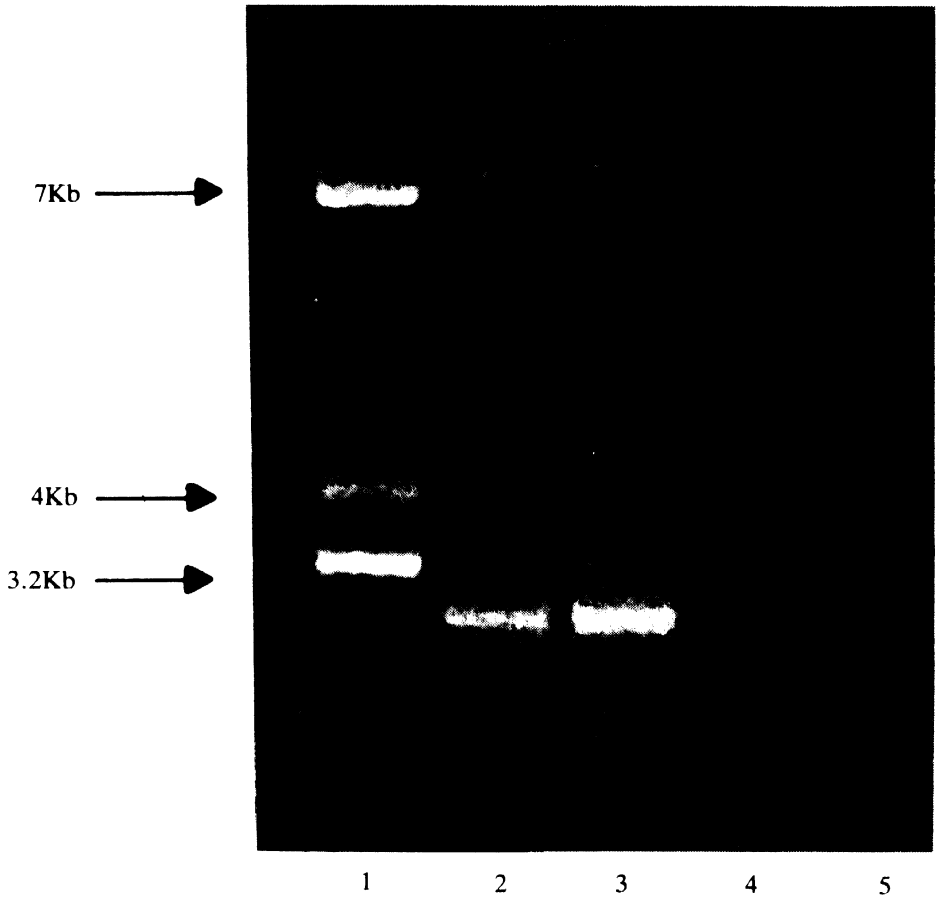

Fig. 5. The results of an agarose gel for the characterisation and the determination of the size of the viral nucleic acid. (1) Three single-stranded, circular DNA markers (5200, 4000 and 3200 bases), (2) the virus nucleic acid, untreated, (3) treated with RNase A, (4) treated with DNase I and (5) treated with Nuclease S1.

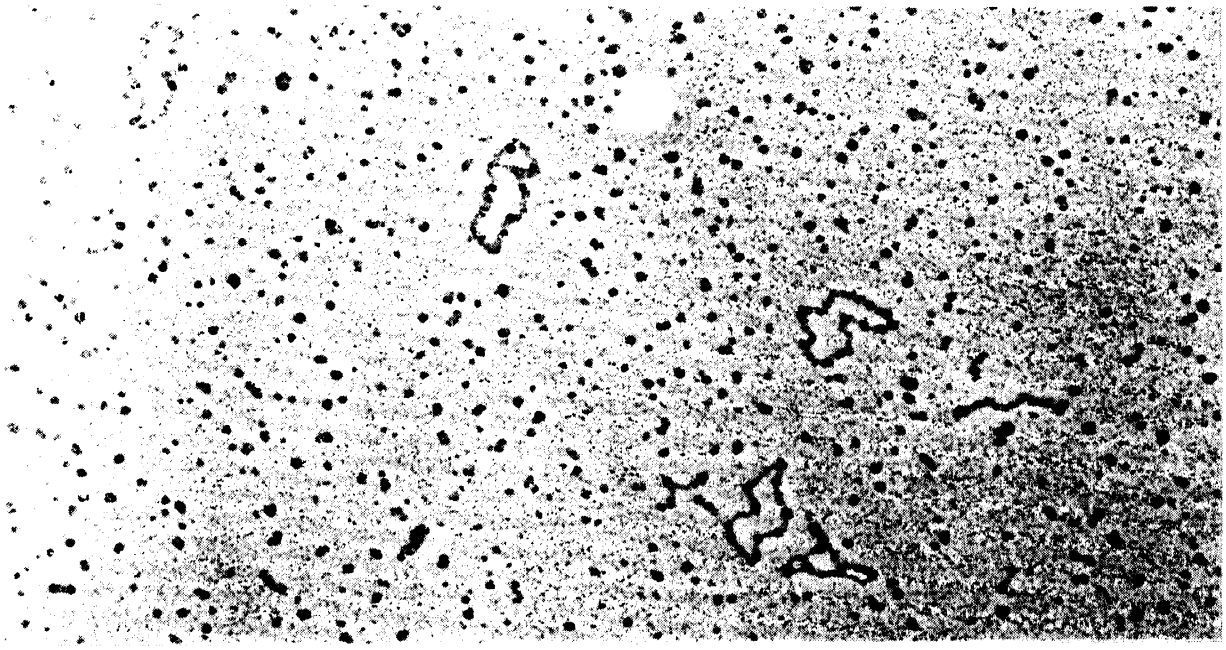

Fig. 6. Electron micrograph of the circular nucleic acid of the chickpea geminivirus.

by the alkaline conditions (pH 9.8) used for coating the virus in DAC-ELISA. Under those alkaline conditions other epitopes might be exposed than in the case in ISEM. The geminiviruses tested might have some internal epitopes in common while they share no, or hardly any, external epitopes. Moreover, the DNA of the chickpea geminivirus did not hybridise with BCTV DNA (J. Stanley, UK personal communication, 1991). These data show that the chickpea geminivirus is a distinct member of the leafhopper-transmitted geminivirus group. The chickpea virus is transmitted by the leafhopper *Orosius orientalis*. Therefore it apparently belongs to the sub-group 2 (Harrison, 1985) including BCTV, BSDV, TYDV. The chickpea produces the characteristic symptoms of stunting, yellowing, leaf curling and distortion, as all the members in this subgroup.

As is clear from Table 3 the chickpea geminivirus reacts strongly with BSDV antiserum and weakly with TYDV antiserum despite the fact that the BSDV virus antiserum has a lower titre (1/32) than the TYDV antiserum (1/128). These two viruses are considered to be strains of the same virus (Thomas & Bowyer, 1980). This finding may suggest that BSDV is more closely related to the chickpea geminivirus than TYDV and might even imply that BSDV and TYDV are more distantly related than they were originally thought to be (Thomas & Bowyer, 1980).

The chickpea virus could infect species of the Leguminosae, the Solanaceae and the Chenopodiaceae (Table 3). Symptoms induced in some hosts by the chickpea virus differ from those produced by BCTV on these hosts (Bennet, 1971; Thomas & Mink, 1979), TYDV (Thomas & Bowyer, 1984; Hill, 1950) and BSDV (Bowyer & Atherton, 1971) (Table 4). The chickpea geminivirus causes only mild or no symptoms on sugarbeet and tomato, respectively, whereas BCTV, BSDV and TYDV cause clear symptoms on these

Table 4. *Comparison of the host range and other characteristics of the chickpea geminivirus, BCTV (Bennet, 1971; Thomas & Mink, 1979), TYDV (Hill, 1950; Thomas & Bowyer, 1984) and BSDV (Bowyer & Atherton, 1971), na = not available*

	Chickpea virus	BCTV	TYDV	BSDV
Beta vulgaris	chlorosis, mild symptoms	chlorosis, leafrolling, severe symptoms	stunting	stunting, reddening
Cucumis sativus	not infected	seedlings killed, dwarfing leafrolling	na	na
Lycopersicon esculentum	symptomless infection	leaf twisting, leafrolling, plant dies	infected, symptoms na	chlorosis downward curling leaflets
Nicotiana tabacum	leaf dwarfing, leaf rolling, plant stunting, no recovery	leaf dwarfing, leaf rolling, recovery	leaf rolling, plant stunting	na
Datura stramonium	chlorosis, leaf rolling, severe plant stunting, plant dies	infected, symptoms na	chlorosis, leaf rolling, slight plant stunting	chlorosis, leaf rolling, slight plant stunting
Nicotiana rustica	not infected	infected, symptoms na	symptomless infection	infected, symptoms na
Coat protein	32 kD	32 kD + 36 kD	27.5 kD	na
Vector	*Orosius orientalis*	*Circulifer tenellus*	*Orosius argentatus*	*Orosius argentatus*

hosts. Moreover, the chickpea virus does not infect *Nicotiana rustica* and *Cucumis sativ* whereas the other viruses do (Table 4). On the other hand, the chickpea virus causes mo severe symptoms on *Datura stramonium* and *Nicotiana tabacum*. These differences i host range support our conclusion based on serology and hybridisation that the chickpe geminivirus is a distinct virus.

Moreover, the coat protein of TYDV (27.5 kD, Thomas & Bowyer, 1980) is considerabl smaller than that of the chickpea virus (32 kD). The size of the coat protein of BSDV i not known since this virus is very unstable. On the basis of host range and serologica relationships the chickpea virus is considered to be a distinct leafhopper-transmitted gem inivirus. Although it appears to be strongly serologically related to BSDV in DAC-ELISA no clear relationship was found with this virus in ISEM. Moreover, the symptoms cause by these two viruses are considerably different and BSDV is unstable (Thomas & Bowyer 1980) whereas the chickpea virus is very stable. On the basis of the characteristic symptom produced by this virus in chickpea, the virus was named chickpea chlorotic dwarf viru (CCDV). CCDV seems to be restricted to the cool-season legumes: pea, chickpea, fab bean and lentil (Table 1). None of the tropical legumes were infected. Since *O. orientalis* breeds on faba bean and lentil, the occurrence of CCDV in these two crops could be important and should be further investigated.

Interestingly, the symptoms caused by CCDV are similar to those produced by the luteovirus causing the chickpea stunt disease (Nene *et al.*, 1991; Nene & Reddy, 1987). In our surveys CCDV was found to be widely distributed in India. We are currently assessing its economic importance in chickpea.

Acknowledgements

The senior author is greatly indebted to the Directorate General for Development Cooperation (DGIS) of the Netherlands Ministry of Foreign Affairs and to the Director General of ICRISAT for providing financial support during his period of research at ICRISAT. We would like to thank Drs Y. L. Nene and D. McDonald for their valuable comments and support. We are grateful to Dr P. Narayana Swamy of the Tamil Nadu Agricultural University, Coimbatore for providing us with a culture of *Orosius orientalis* and to Dr Viraktamath, University of Agricultural Sciences, Bangalore, and the International Institute of Entomology, CAB International, UK, for confirming its identity. We would like to thank Drs K. R. Bock, J. E. Duffus, R. I. B. Francki, B. D. Harrison, K. Lindsten, D. L. Mumford and J. E. Thomas for their generous gifts of antisera and Dr J. Stanley (John Innes Institute, UK) for providing the results of hybridisation with BCTV DNA, Dr D. J. Robinson and G. H. Duncan (SCRI, UK) for providing the electron micrograph of the viral DNA and to Mr A. K. Murthy (ICRISAT) for help with the electron microscopy.

References

Bennet C W. 1971. The curly top disease of sugarbeet and other plants. *The American Phytopathological Society. Monograph No. 7.*

Bosque-Perez N A, Buddenhagen I W. 1990. Studies on epidemiology of virus diseases of chickpea in California. *Plant Disease* **74**:372–378.

Bowyer J W, Atherton J G. 1971. Summer death of French bean: new hosts of the pathogen, vector relationships and evidence against mycoplasmal etiology. *Phytopathology* **61**:1451–1455.

Clark M F, Adams A N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* **34**:475–483.

Duffus J E. 1979. Legume yellows virus, a new persistent aphid-transmitted virus of legumes in California. *Phytopathology* **69**:217–221.

Goodman R M, Bird J. 1978. Bean golden mosaic virus. *CMI/AAB Descriptions of Plant Viruses No. 192*. 4 pp.

Harrison B D. 1985. Advances in geminivirus research. *Annual Review of Phytopathology* **23**:55–82.

Hill A V. 1950. Yellow dwarf of tobacco in Australia. *Australian Journal of Agricultural Research* **1**:141–143.

Hobbs H A, Reddy D V R, Rajeshwari R, Reddy A S. 1987. Use of direct antigen coating and protein A coating ELISA procedures for detection of three peanut viruses. *Plant Disease* **71**:747–749.

Kaiser W J, Danesh D. 1971. Etiology of virus-induced wilt of *Cicer arietinum*. *Phytopathology* **61**:453–457.

Laemmli U K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T-4. *Nature* **227**:680–685.

Murant A F, Taylor M, Duncan G M, Raschke J H. 1981. Improved estimates of molecular weight of plant virus RNA by agarose gel electrophoresis and electron microscopy after denaturation with glyocal. *Journal of General Virology* **53**:321–332.

Nene Y L, Reddy M V. 1987. Chickpea diseases and their control. In *The Chickpea III viral diseases*, pp. 233–270. Eds M C Saxena and K B Singh. CAB International, ICARDA.

Nene Y L, Reddy M V, Haware M P, Ghanekar A M, Amin K S. 1991. Field diagnosis of chickpea diseases and their control. *Information Bulletin No. 28*, ICRISAT.

Reddy M V, Nene Y L, Verma J P. 1979. Pea leaf roll virus causes chickpea stunt. *International Chickpea Newsletter* **1**:8.

Reddy D V R, Sudarshana M R, Ratna A S, Kiran Kumar I, Murthy A K. 1990. The occurrence of yellow spot virus, a new member of the tomato spotted wilt virus group, on peanut (*Arachis hypogaea* L.) in India. In *Proceedings of tomato spotted wilt virus workshop, Beltsville, Maryland, USA: USDA*, pp. 77–83.

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.

Roberts I M, Robinson D J, Harrison B D. 1984. Serological relationships and genome homologies among geminiviruses. *Journal of General Virology* **65**:1723–1730.

Singh S, Nagaich B B, Bhargawa K S. 1983. *Orosius albicinctus* and the potato purple top roll: vector pathogen relationship. *Journal of the Indian Potato Association* **10**:34–41.

Sudarshana M R, Reddy D V R. 1989. Penicillinase based enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of Virological Methods* **26**:45–52.

Thomas J E, Bowyer J W. 1980. Properties of tobacco yellow dwarf and bean summer death viruses. *Phytopathology* **70**:214–217.

Thomas J E, Bowyer J W. 1984. Tobacco yellow dwarf virus. *CMI/AAB Descriptions of Plant Viruses No. 278*. 4 pp.

Thomas J E, Mink G I. 1979. Beet curly top virus. *CMI/AAB Descriptions of Plant Viruses No. 210*. 6 pp.

Van den Heuvel J F J M, de Blank C M, Goldbach R W, Peters D. 1990. A characterization of epitopes on potato leafroll virus coat protein. *Archives of Virology* **115**:185–197.

Vasudeva R S, Sahambi H S. 1955. Phyllody in sesamum (*Sesamum orientale* L.). *Indian Phytopathology* **8**:124–129.

Vieira J, Messing J. 1987. Production of single-stranded plasmid DNA. In *Methods in Enzymology* Vol. 153, pp. 3–11. Eds R Wu and L Grossman.

(Received 27 July 1992)