Etiology

Indian Peanut Clump Virus Isolates: Host Range, Symptomatology, Serological Relationships, and Some Physical Properties

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ABSTRACT

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The symptomatology of Indian peanut clump virus (IPCV) isolates collected from five different geographical locations, Bapatla (B), Chinnaganjam (C), Hyderabad (H), Ludhiana (L), and Talod (T), differed. B-IPCV and C-IPCV were indistinguishable by host range but could be distinguished from the other isolates by symptoms on *Canavalia ensiformis, Nicotiana clevelandii* × *glutinosa, Phaseolus vulgaris*, and *Vigna unguiculata*. B-IPCV, C-IPCV, and T-IPCV were related serologically, but could be distinguished from H-IPCV and L-IPCV isolates in serological tests. The five isolates could not be distinguished on the basis of particle size. Each isolate contained two RNA species of 1.90×10^6 and 1.65×10^6 M_r estimated under nondenaturing conditions and a single polypeptide of 24×10^3 M_r. Significance of these findings for the diagnosis of IPCV and for screening of peanut genotypes for resistance is discussed.

Peanut clump, a soilborne virus disease, was first reported on peanut (*Arachis hypogaea* L.) in West Africa (16). The causal virus was shown to be rod-shaped (13) with two predominant particle lengths, 190 and 245 nm, containing two RNA species of 1.7×10^6 and 2.1×10^6 M_r (15). Reddy et al (9) have reported a similar disease on peanuts from the Punjab State, India, caused by a virus with morphologically similar particles. Because the Indian virus was serologically unrelated to the West African virus, it was named Indian peanut clump virus (IPCV) (9). During disease surveys in various peanut-growing locations in India a similar disease was observed. However, there were differences in the severity of stunting and clumping on the same susceptible peanut cultivars. Selection and breeding strategies for IPCV resistance depended on whether the variability in symptom expression between geographical locations was the result of edaphic or climatic effects on a single virus isolate or caused by the presence of different strains of IPCV.

This paper reports a comparison of symptomatology, host range, serological relationships, and viral polypeptide and nucleic acid molecular weights for five isolates of IPCV from different geographical regions.

MATERIALS AND METHODS

Collection and maintenance of isolates. The five IPCV isolates used in this study were collected in different locations in India and maintained in the peanut cultivar M-13 by sap inoculations, as described by Reddy et al (9). Isolates were designated according to the collection location: B-IPCV from Bapatla, Andhra Pradesh; C-IPCV from Chinnaganjam, Andhra Pradesh; H-IPCV from Hyderabad, Andhra Pradesh; L-IPCV from Ludhiana, Punjab; and T-IPCV from Talod, Gujarat. A single lesion isolate of C-IPCV was obtained by five successive single lesion transfers in *Chenopodium quinoa* Willd. The other four isolates were passed five consecutive times through single lesions in cowpea (*Vigna*

unguiculata (L.) Walp. subsp. *unguiculata* 'Early Ramshorn'). Each isolate was maintained in bean (*Phaseolus vulgaris* L. 'Top Crop').

Host range. Six plants of each of 14 species were inoculated and grown in a screenhouse where temperatures ranged between 20–30 C. Local or systemically infected tissue from test plants were inoculated to bean or *C. quinoa* after 11–16 days.

Virus purification. Each isolate was propagated in Top Crop bean. Infected primary leaves exhibiting characteristic symptoms were homogenized in 0.1 M phosphate buffer, pH 8.0, containing 1% 2-mercaptoethanol and 0.01 M sodium diethyldithiocarbamate (4 ml of buffer per gram of leaves). The homogenate was filtered through cheesecloth, adjusted to 10% chloroform (v/v), shaken for 10 min, and clarified at 3,000 *g* for 10 min. Virus in the aqueous phase was precipitated by adding polyethylene glycol (PEG) to 6% and NaCl to 0.2 M. After a 1.5–2-hr incubation at 4 C the precipitate was collected at 10,000 *g* for 10 min; resuspended in 0.01 M borate-phosphate buffer, pH 8.3, containing 0.2 M urea; and clarified at 5,000 *g* for 10 min. Triton X-100 was added to the supernatant to 0.5%. Subsequent steps in the purification were described by Reddy et al (10).

Production of antiserum. Purified virus of each isolate was suspended in 0.01 M potassium phosphate buffer, pH 7.0, adjusted to a concentration of 1 mg/ml, emulsified with an equal volume of Freund's incomplete adjuvant, and injected intramuscularly into New Zealand white rabbits at weekly intervals for 4 wk. Serum was collected a month after the final injection. Homologous antisera titers determined in the precipitin ring interface test were: B-IPCV (1/1,280), C-IPCV (1/2,500), H-IPCV (1/640), L-IPCV (1/1,280), and T-IPCV (1/640).

Serology. The direct double-antibody sandwich form of enzyme-linked immunosorbent assay (DAS-ELISA) and immunosorbent electron microscopy (ISEM) with or without decoration were employed to compare the serological relationships among isolates. The DAS-ELISA procedure followed was described earlier (7). Coating globulin was used at a concentration of 1 to 2 μg/ml. A 1/100 sap dilution was used. Alkaline phosphatase conjugated globulins for homologous reactions were used at 2 to 3 μg/ml and globulins for heterologous reactions were used at 10–20 μg/ml. Absorbance values of at least two times that of healthy peanut extracts were considered positive. For ISEM, Formvar-coated, carbon-stabilized 200-mesh copper grids were floated on 10-μl drops of protein A (100 μg/ml) for 1 hr at 37 C, washed with phosphate buffer, and placed on 50-μl drops of antiserum (1/100 dilution) 1 hr at 37 C. Grids were washed with phosphate buffer and floated overnight at 4 C on drops of crude extracts of bean leaves diluted to 1/5 with phosphate buffer. Grids were subsequently washed with 30–40 drops of distilled water and stained with 2% aqueous uranyl acetate for 2 min. The number of virus particles trapped by each antiserum was determined by counting the total number of virus particles in 10 random viewing fields at a magnification of 45,000×. The amount of trapping by each antiserum was expressed as an increase factor over the number of particles on the corresponding preimmune serum-coated grid (11). ISEM with decoration was similar to that described by Milne and Luisoni (5). Virus particles were trapped on the grid surface, as described above. Grids were then incubated for 1 hr at 37 C on drops of antiserum diluted to 1/250 with phosphate buffer, washed and stained, and the relative amount of decoration was assessed at magnifications of 45,000 and 70,000×. Grids incubated on preimmune serum served as negative decoration controls.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of viral coat protein. Purified virus subjected to two cycles of sucrose rate-zonal gradient centrifugation was solubilized by resuspending in disruption buffer and heating at 100 C for 2 min (8). Electrophoresis was performed on a 10% resolving gel with a 4% spacer gel using the Laemmli discontinuous buffer system (4). Gels were stained with Coomassie Blue R-250 and the relative molecular mass of the viral coat protein estimated using SDS-PAGE low molecular weight standards (Bio-Rad, Richmond, CA).

Polyacrylamide gel electrophoresis of viral RNA. Viral RNA was extracted as described (2). Electrophoresis was performed in 0.036 M Tris, 0.03 M NaCl, and 0.001 M disodium EDTA buffer, pH 7.8, on a 12 × 14-cm composite slab gel containing 2.1% polyacrylamide and 0.5% agarose. Following electrophoresis for 4 hr at 50 V the gels were stained with ethidium bromide (1 μg/ml) for 1 hr and destained in distilled water. Gels were viewed using a spectroline TR 302 UV transilluminator. *Escherichia coli* ribosomal RNAs 16 S (0.53×10^6 M_r) and 23 S (1.07×10^6 M_r) and tobacco mosaic virus RNA (2.01×10^6 M_r) were used as standards.

RESULTS

Host range. At least 10 of the 15 inoculated host range plants including *A. hypogaea, Canavalia ensiformis* (L.) DC., *C. amaranticolor* Costa & Reyn., *C. murale* L., *C. quinoa, Nicotiana clevelandii* Gray × *glutinosa* L. hybrid, *P. mungo* L., *P. vulgaris* L. ('Local' and 'Top Crop'), *Vicia faba* L., and *V. unguiculata* (L.) Walp. subsp. *unguiculata* ('C-152' and 'Early Ramshorn') were infected by at least one of the five IPCV isolates tested. *Nicotiana bigelovei* Gray and *N. rustica* L. were not infected by any of the isolates tested. All five isolates produced identical symptoms on *A. hypogaea, P. mungo*, and *V. faba*. Five host range plants were selected on which the five IPCV isolates produced a differential symptom response (Table 1). The B-IPCV and C-IPCV isolates produced similar symptoms on all host range plants, whereas the L-IPCV, H-IPCV, and T-IPCV isolates each produced a unique set of symptoms. *C. ensiformis* and *N. clevelandii* × *glutinosa* hybrid were the most useful hosts for separating isolates on the basis of symptom expression.

Serological comparison of isolates. The antigen and conjugate concentrations used in DAS-ELISA were optimal for the detection of weak serological cross reactions. A 1/100 sap dilution reduced any nonspecific reaction in healthy peanut extracts to the level of the buffer control. Although not essential for the detection of differences, a higher conjugate concentration in heterologous combinations slightly enhanced weak cross reactions. The B-IPCV, C-IPCV, and T-IPCV isolates were determined to be serologically similar in DAS-ELISA tests, but not related to the L-IPCV and H-IPCV isolates (Table 2). L-IPCV and H-IPCV antisera reacted only with homologous antigens in DAS-ELISA.

TABLE 1. Symptomatology of five host species inoculated with five isolates of Indian peanut clump virus (IPCV)

	Reactions to IPCV isolates			
Host range plants	B-IPCV[a] C-IPCV	L-IPCV	H-IPCV	T-IPCV
Canavalia ensiformis	Large necrotic patches	Small necrotic lesions	Symptomless	Large chlorotic patches
Nicotiana clevelandii × *glutinosa*	Mild mottle	Severe mosaic	Symptomless	Not infected
Phaseolus vulgaris 'Top Crop'	Veinal chlorosis	Veinal necrosis	Extensive veinal necrosis	Veinal chlorosis
Vigna unguiculata subsp. *unguiculata* 'Early Ramshorn'	Chlorotic local lesions	Chlorotic ringspots	Chlorotic local lesions	Chlorotic local lesions
V. unguiculata subsp. *unguiculata* 'C-152'	Chlorotic local lesions	Chlorotic local lesions	Chlorotic local lesions	Chlorotic ringspots

[a] Sources of isolates: B = Bapatla, Andhra Pradesh; C = Chinnaganjam, Andhra Pradesh; L = Ludhiana, Punjab; H = Hyderabad, Andhra Pradesh; and T = Talod, Gujarat.

TABLE 2. Determination of the serological relationship between five Indian peanut clump virus (IPCV) isolates using direct double-antibody sandwich form of enzyme-linked immunosorbent assay

Coating and conjugate antibodies	Antigen[a]					Healthy peanut extract
	B-IPCV[b]	C-IPCV	H-IPCV	L-IPCV	T-IPCV	
B-IPCV	0.385[c]	0.362	0.020	0.050	0.123	0.070
C-IPCV	0.299	0.293	0.060	0.040	0.290	0.030
H-IPCV	0.090	0.060	0.460	0.090	0.080	0.030
L-IPCV	0.060	0.030	0.030	0.364	0.040	0.090
T-IPCV	0.380	0.375	0.020	0.060	0.410	0.060

[a] Leaf extracts from infected peanut plants, employed at 1:100 dilution based on fresh weight.
[b] Sources of isolates: B = Bapatla, Andhra Pradesh; C = Chinnaganjam, Andhra Pradesh; H = Hyderabad, Andhra Pradesh; L = Ludhiana, Punjab; and T = Talod, Gujarat.
[c] The mean A_{405nm} value for triplicate wells.

TABLE 3. Serological comparison of five Indian peanut clump virus (IPCV) isolates using immunosorbent electron microscopy trapping

Trapping antibody	Virus isolate[a]				
	B-IPCV[b]	C-IPCV	H-IPCV	L-IPCV	T-IPCV
B-IPCV	172[c]	103	2	2	7
C-IPCV	259	155	2	2	203
H-IPCV	2	2	6	2	2
L-IPCV	2	2	4	60	2
T-IPCV	204	113	2	2	115

[a] Virus particles were trapped from Top Crop bean leaf extracts diluted 1:5 in 0.01 M potassium phosphate buffer, pH 7.0.
[b] Sources of isolates: B = Bapatla, Andhra Pradesh; C = Chinnaganjam, Andhra Pradesh; H = Hyderabad, Andhra Pradesh; L = Ludhiana, Punjab; and T = Talod, Gujarat.
[c] The amount of trapping expressed as a ratio calculated by dividing the number of virus particles counted on an antiserum-coated grid by the number of virus particles counted on the corresponding preimmune serum-coated grid. Virus particles were counted in 10 random viewing fields at 45,000× for each antibody:virus combination. Values greater than 2 indicate a serological relationship. The total number of particles trapped for each homologous antibody:virus combination and preimmune serum control were: B-IPCV (1,204 and 7); C-IPCV (1,860 and 12); H-IPCV (72 and 12); L-IPCV (360 and 6); and T-IPCV (1,268 and 11).

TABLE 4. Serological comparison of five Indian peanut clump virus (IPCV) isolates using immunosorbent electron microscopy decoration

Decorating antibodies	Relative amount of virus particle decoration between IPCV isolates[a]				
	B-IPCV[b]	C-IPCV	H-IPCV	L-IPCV	T-IPCV
B-IPCV	4[c]	3	0	0	3
C-IPCV	2	3	0	0	2
H-IPCV	0	0	2	3	0
L-IPCV	0	0	3	0	0
T-IPCV	1	3	0	0	3

[a] Virus particles were trapped on the grid surface from Top Crop bean leaf extract using homologous antiserum. The decorating antibodies were diluted 1/250 with 0.01 M potassium phosphate buffer, pH 7.0, and incubated on the grids for 1 hr at 37 C.
[b] Sources of isolates: B = Bapatla, Andhra Pradesh; C = Chinnaganjam, Andhra Pradesh; H = Hyderabad, Andhra Pradesh; L = Ludhiana, Punjab; and T = Talod, Gujarat.
[c] The amount of decoration observed was expressed on a numerical scale from 0 to 4 with 4 representing the heaviest amount of decoration observed. Grids incubated with preimmune serum served as zero decoration controls.

Similar serological relationships were observed using ISEM (Table 3). A strong serological relationship existed between the B-IPCV, C-IPCV, and T-IPCV isolates. Although B-IPCV antiserum was inefficient in trapping T-IPCV virus particles, the increase factor in the reciprocal reaction using T-IPCV antiserum was high, indicating T-IPCV should be grouped with B-IPCV and C-IPCV. There was a small increase in the number of H-IPCV particles trapped by L-IPCV antiserum, but this result could not be confirmed in the reciprocal test using H-IPCV antiserum to trap L-IPCV particles.

Results from ISEM with decoration confirmed the close serological relationship between B-IPCV, C-IPCV, and T-IPCV isolates (Table 4). Heavy particle decoration (Fig. 1C) was observed in reciprocal tests with antisera to these three isolates. Light decoration (Fig. 1B) of L-IPCV particles by H-IPCV antibodies was observed; however, L-IPCV antibodies failed to decorate (Fig. 1A) H-IPCV particles in numerous attempts. Heavy decoration was consistently observed in all homologous antibody:virus combinations.

Comparison of the protein and nucleic acid relative molecular mass. The single viral polypeptide for all isolates was estimated to be 24×10^3 M_r. The two nucleic acid species were estimated to be 1.90×10^6 and 1.65×10^6 M_r for all the isolates.

DISCUSSION

Results presented demonstrate that IPCV occurs as isolates differing in symptomatology and serological properties. B-IPCV and C-IPCV were shown to be very similar on the basis of host range and serology. The only difference noted between these two isolates was the weaker reaction observed in ELISA and ISEM between B-IPCV antiserum and T-IPCV particles. This difference may be useful in identifying these isolates. The H-IPCV, L-IPCV, and T-IPCV isolates could all be distinguished from one another and from B-IPCV and C-IPCV by symptom expression. Although T-IPCV was distinct on the basis of host range it was serologically related to the B-IPCV and C-IPCV isolates. Although the H-IPCV and L-IPCV isolates were distinguishable by host range, they were also serologically distinct in DAS-ELISA, which has been shown to detect narrow serological relationships (3). The apparent serological reaction between H-IPCV and L-IPCV observed in ISEM and decoration tests could not be confirmed in reciprocal combinations. The failure to demonstrate a serological relationship between H-IPCV and L-IPCV in ISEM is consistent with that reported by Reddy et al (10). However, strong nucleic acid homologies were observed among B-IPCV, H-IPCV, and L-IPCV (10). A similar situation exists with tobacco rattle virus (TRV) isolates, which have a high degree of nucleotide sequence homology in RNA-1 with little or no detectable antigenic relationship (1). However, TRV differs from IPCV in particle size and has a nematode vector (1) instead of a fungal vector. PCV from West Africa was shown to be transmitted by *Polymyxa graminis* (14), and IPCV also appears to be transmitted by *P. graminis* (6). Based on evidence presented in this paper and by Reddy et al (10) IPCV should be included in the furovirus group recently proposed by Shirako and Brakke (12) to include fungus-transmitted viruses with rod-shaped particles. Among furoviruses, only IPCV has so far been shown to exist as isolates distinguished by serology and host range.

All the IPCV isolates, with the exception of B-IPCV and C-

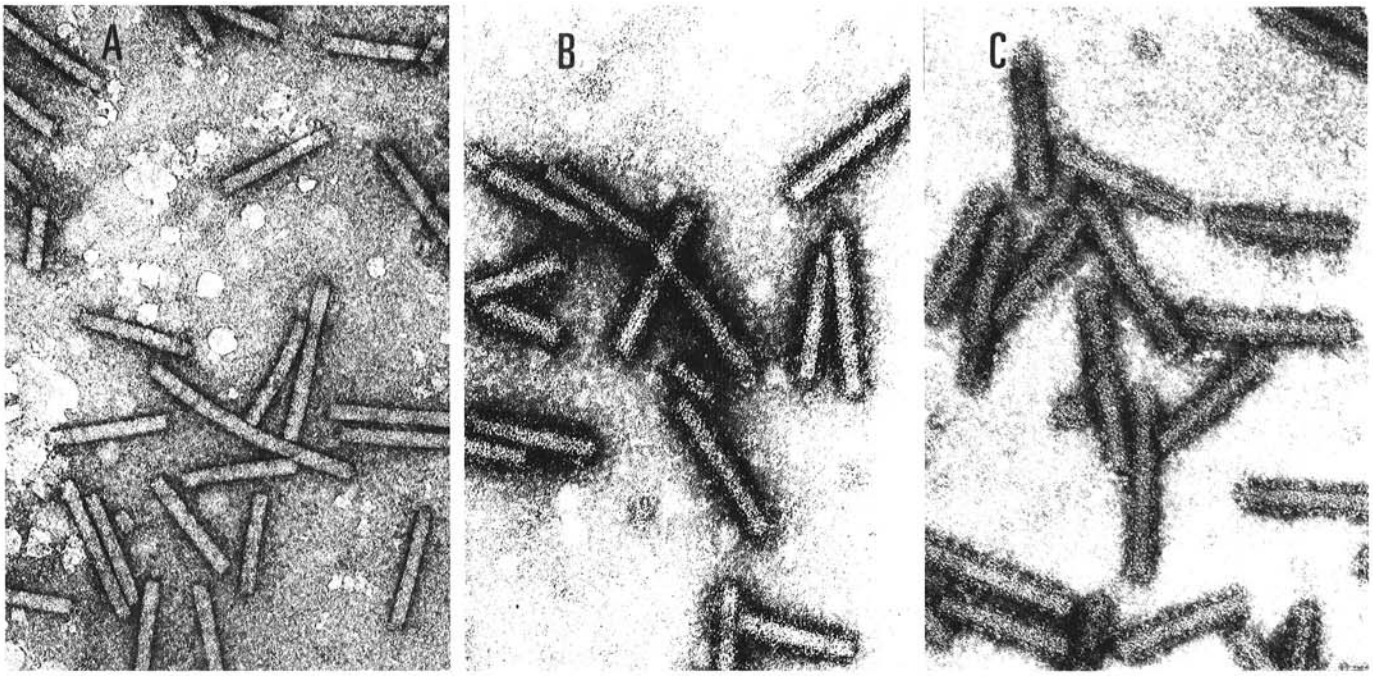

Fig. 1. The range of decoration observed among different IPCV antisera:virus combinations. **A,** No decoration. **B,** Light decoration. **C,** Heavy decoration.

IPCV, were collected at least 350 km apart. In our preliminary tests, peanut genotypes resistant to L-IPCV in the Ludhiana region were susceptible when evaluated in the Bapatla region. Therefore, the screening of peanut genotypes for resistance to IPCV will have to be done at different locations, and the development of alternative control methods to complement breeding for resistance will be an essential component in the management of the peanut clump disease in India. Because nucleic acid hybridization tests demonstrated extensive sequence homology among serologically unrelated isolates (10), cDNA hybridization probes may be more reliable for detecting the presence of IPCV isolates than the use of serology. Work on improved virus detection and disease management practices is now in progress.

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