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# THE NUCLEOTIDE SEQUENCE OF INDIAN PEANUT CLUMP VIRUS RNA 2

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*Summary*

The RNA-2 molecule of an isolate of the L serotype of Indian peanut clump virus (IPCV) is 4290 nucleotides in length and contains five open reading frames (ORF). The arrangement of the ORFs is similar to that in RNA-2 of peanut clump virus (PCV) from West Africa. Proteins encoded by IPCV-L RNA-2 are between 32% and 89% identical to those of PCV. The coat protein of IPCV-L is as similar to the corresponding coat proteins of isolates belonging to H and T serotypes of IPCV as they are to the coat protein of PCV. The results support the distinction of IPCV-L and PCV as separate virus species, although there are strong similarities among triple gene block proteins.

*Introduction*

Peanut clump is an economically important soil-borne virus disease of peanuts (*Arachis hypogaea* L) caused by Indian peanut clump virus (IPCV) in India (Reddy *et al.*, 1983) and peanut clump virus (PCV) in West Africa (Thouvenel and Fauquet, 1981). Particles of both viruses are rod-shaped, of two predominant lengths and contain two genomic RNAs; they have been classified in the genus *Furovirus*. The currently known isolates of IPCV fall into three serotypes, viz. IPCV-H, IPCV-L and IPCV-T (Nolt *et al.*, 1988) and each is serologically distinct from PCV.

The nucleotide sequence of the two genomic RNAs of an isolate of PCV is known (Manohar *et al.*, 1993; Herzog *et al.*, 1994). The nucleotide sequence of the coat protein gene of IPCV-H (Wesley *et al.*, 1994) showed the coat protein to be 61% identical in amino acid sequence to that of the PCV, which suggests that IPCV and PCV are different viruses. However, attempts to clone and sequence remainder of IPCV-H RNA-2 were not successful. In order to extend comparisons between IPCV and PCV, we have determined the nucleotide sequences of all of IPCV-L RNA-2 and of the coat protein gene of isolate D, which was collected by P. Delfosse in Durgapura, Rajasthan in 1994 and belongs to serotype T.

*Materials and Methods*

*Purification of virus and extraction of RNA*

The IPCV-L was purified as described by Reddy *et al.* (1985). RNA was extracted from purified virus particles and RNA 2 was separated from RNA 1 in low melting agarose gels.

*Cloning and sequencing*

RNA-2 was heat denatured at 65°C for 5 min, annealed with random composition hexa-deoxynucleotides and used as a template for cDNA synthesis. The ds cDNA was cloned in pUC119. Nucleotide sequencing was done by dideoxy chain termination using Sequenase (Amersham). The extremities of the RNA-2 were cloned using the 5'Amplifinder™ RACE Kit (Clontech). All the sequences were determined in both directions. Nucleotide sequences were compared by using programs DIAGON (Staden, 1982) and CLUSTALV (Higgins *et al.*, 1992).

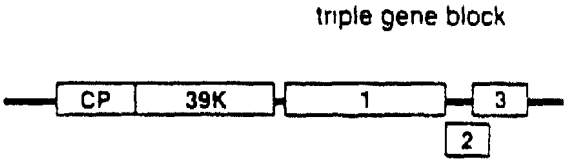


Fig 1 Diagram of the genome organization of IPCV L RNA-2

The IPCV-L RNA-2 is 4290 nucleotides (nt) in length and contains five open reading frames (Fig 1) The coat protein is the 5-most gene and is followed by a $M_r$39000 protein gene (39K) and three overlapping genes at the 3 end which form a triple gene block (TGB) The genome organization of IPCV-L RNA-2 is similar to that of PCV

Fig 2 shows a DIAGON comparison of RNA-2 sequences between IPCV-L and PCV The results suggest that the sequences in the 3 half of the two molecules (which encode the TGB proteins) are more alike than sequences in the 5 half of the molecules (which encode

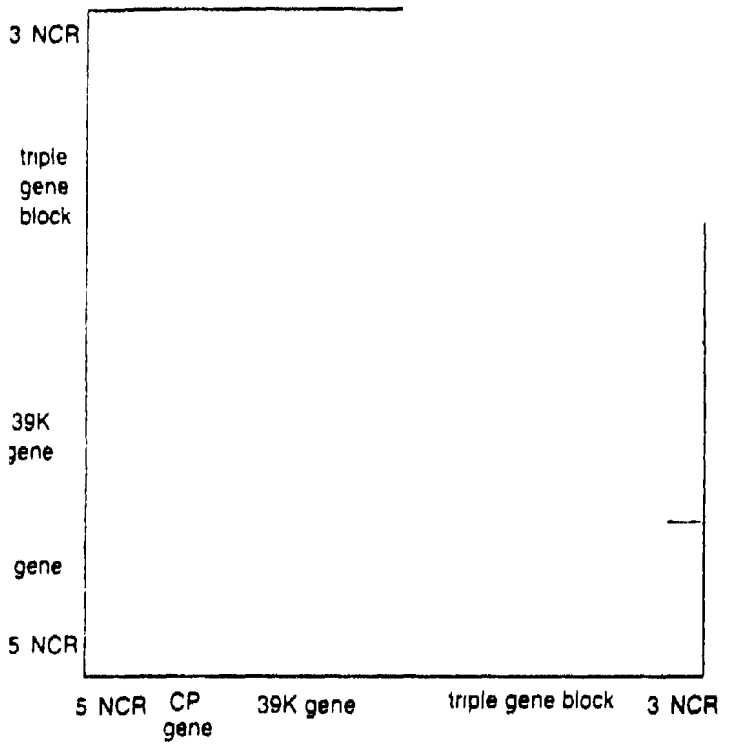


Fig 2 DIAGON comparison of RNA 2 molecules of IPCV L (horizontal) and PCV (vertical) Matches shown are for 11+/15 identical

the coat protein and 39K) Different parts of RNA-2 molecule differ in the extent to which they vary among IPCV-L and PCV the ORF2 of the TGB is highly conserved (89% identical) whereas the 39K is only 32% identical

Analysis by using CLUSTALV of the coat protein sequences of IPCV and PCV suggested that the coat protein of PCV is 59% identical to that of IPCV-H 67% identical to that of IPCV-L and 62% identical to that of IPCV-D The coat proteins of IPCV and PCV were also

compared with corresponding proteins of other viruses. Fig. 3 shows an unrooted tree illustrating the similarity distances between coat protein genes of IPCV serotypes, PCV, BSMV, potato mop-top (PMTV), soil-borne wheat mosaic (SBWMV), beet necrotic yellow vein (BNYVV) and Nicotiana velutina mosaic (NVMV) furoviruses. The only marked clustering detected (shaded in Fig. 3) was between IPCV serotypes and PCV. However, the coat proteins were more similar to that of BSMV than to coat proteins of any other furovirus. Thus, different furoviruses were as dissimilar from each other as they were from IPCV or PCV.

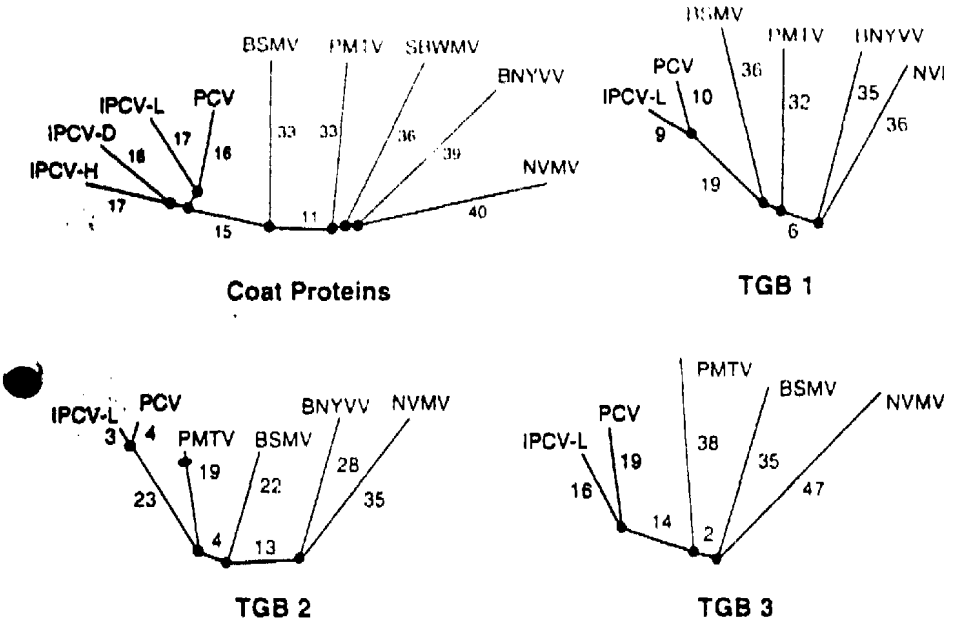


Fig. 3. Unrooted tree showing relationships among coat protein sequences and triple gene block proteins estimated by CLUSTALV

Fig. 3 also shows an analysis of relationships among the TGB proteins. The distances between IPCV-L and PCV are like those between the coat proteins for protein TGB 3 but the other TGB proteins of the two viruses are more alike. The striking difference between comparisons among coat proteins and TGB proteins is that whereas the coat protein of PMTV is distant from that of IPCV, the TGB proteins of PMTV are as close to those of IPCV and PCV as are the BSMV TGB proteins.

*Discussion*

The results show that the RNA-2 of IPCV-L and PCV are distinct, although their genome organizations are similar. The extent to which corresponding genes were similar depended on which genes were compared; the 5'-most genes were more different than those in the 3' half of the RNA-2 molecule. Comparisons among the sequences of the corresponding genes of RNA-1 of IPCV-H (Miller *et al.*, this volume) and PCV (Herzog *et al.*, 1994), and RNA-2 of IPCV-L and PCV (Manohar *et al.*, 1993; Herzog *et al.*, 1994) showed that RNA-2 gene products differed more than RNA-1 products. There is no strong similarity between RNA-1 and RNA-2 of IPCV and PCV except in the 3' non-coding region. The pattern of genome variation in IPCV and PCV is like that in tobacco rattle virus (TRV); RNA-1 molecules of all