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THE NUCLEOTIDE SEQUENCE OF RNA-1 OF INDIAN PEANUT CLUMP VIRUS COMPLICATES ITS TAXONOMY BUT OFFERS BROAD SPECTRUM DIAGNOSTICS

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Summary

The nucleotide sequence of the 5841 nucleotides of RNA-1 of Indian peanut clump virus (IPCV) contains three open reading frames which encode a M_r 129687 protein containing a methyltransferase domain (ORF 1), a M_r 60188 protein which contains a polymerase domain (ORF 2), and a M_r 14281 protein (ORF 3). The nucleotide sequence of IPCV RNA-1 is similar to that of peanut clump virus (PCV). The translation products are 88% (ORF 1), 95% (ORF 2) and 75% (ORF 3) identical to those of PCV RNA and are also similar to those of soil-borne wheat mosaic virus and barley stripe mosaic virus (BSMV). These similarities and those with proteins of other furoviruses call into question the taxonomic status of the genera *Furovirus* and *Hordeivirus*. A cDNA probe derived from the 3' end of RNA-1 of IPCV-H hybridized with RNA of isolates of other serotypes of IPCV and of PCV. Also, primers based on sequences common to RNA-1 of IPCV and PCV, were effective in reverse transcription-PCR amplification of these RNA and that of L and T serotype isolates of IPCV.

Introduction

Indian peanut clump virus (IPCV) induces clump disease in groundnut crops in many parts of India, and is transmitted by *Polymyxa graminis* (Reddy *et al*., 1988). IPCV resembles peanut clump virus (PCV), which induces clump disease of groundnut crops in West Africa (Thouvenal and Fauquet, 1981). There are three serotypes of IPCV (H, L or T) (Nolt *et al*., 1988) and this serological diversity is a potential complication for disease diagnosis. IPCV has a bi-partite genome of positive-sense RNA molecules encapsidated in rod-shaped particles of different lengths (Reddy *et al*., 1985); it is classified in the genus *Furovirus*.

Materials and methods

Virus isolates and propagation:
Hyderabad (H) and Ludhiana (L) isolates, which belong to the H and L serotypes (Nolt *et al*., 1988), and the D isolate, collected by P. Delfosse in 1994 from Durgapura, Rajasthan (serotype T), were propagated in *Phaseolus vulgaris* and purified as described by Reddy *et al*. (1985).

Nucleotide sequencing:
RNA was extracted from purified virus particles and cDNA was synthesized using a commercial kit (Boehringer) and cloned in *Sma* I - digested pUC19. The 5'- and 3'-extremities of IPCV RNA-1 were cloned using the 5'Amplifinder RACE kit (Clontech).

Nucleotide sequences were determined by dideoxy chain termination using Sequenase (Amersham) and were compared using DIAGON (Staden, 1982) or CLUSTALV (Higgins *et al*., 1992).

RT-PCR:
The locations of the primers chosen which matched the sequences of IPCV RNA-1 and PCV

RNA-1 (Herzog *et al.*, 1994) with minimum degeneracy are shown in Fig. 1. Template was 0.5 μg of virus RNA or 5 μg of RNA extracted from the leaf tissue of IPCV-infected *Nicotiana benthamiana*. Reverse transcription at 42°C for 1 hour was followed by 30 cycles of PCR at 94°C for 1 min, 55°C for 1 min, 72°C for 2 min.

Results

A sequence of 5841 nucleotides was established. It contained 3 open reading frames

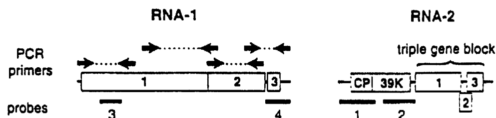

Fig. 1. Diagram of the genome of Indian peanut clump virus

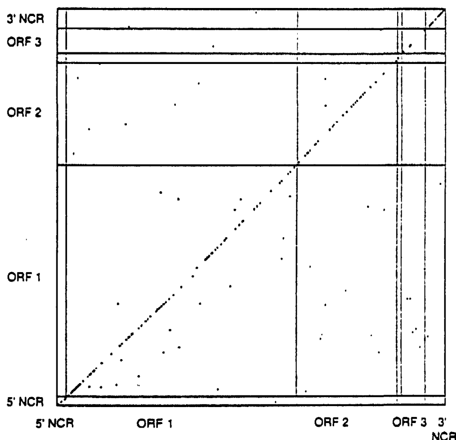

Fig. 2. DIAGON comparison of RNA-1 molecules of IPCV-H (horizontal) and PCV (vertical). Matches shown are for 11+/15 nucleotides identical.

(ORF). ORF 1 has domains suggestive of methyltransferase activity. ORF 2 has a polymerase domain but ORF 3 did not resemble proteins of known function. The genome organization (Fig.1) is very like that of PCV (Herzog *et al*., 1994).

Comparison of the two sequences using DIAGON recording matching strings in which 11 of 15 nucleotides were identical (Fig. 2) showed a non-uniform distribution of similarity. The non-coding regions (NCR) were the most alike, there were patches of strong similarity in ORF1 and ORF2. and ORF3 were least alike.

Fig. 3 shows the result of comparisons, made using CLUSTALV, among the proteins

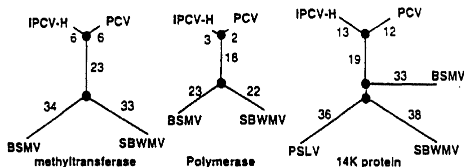

Fig. 3. Relatedness among corresponding proteins assessed by CLUSTALV

encoded by each of the ORF and the corresponding proteins of PCV and other viruses, when any perceptible similarities could be discerned.

Fragments of cDNA corresponding to different parts of the IPCV genome were tested in hybridization tests with RNA of each of the three IPCV serotypes as well of PCV RNA. The result (Table 1) was that probes from RNA-2 were relatively specific for the homologous H

	probe 1	probe 2	probe 3	probe 4
IPCV-H	++++	+++	+++	++++
IPCV-L	+/-	-	++	++++
IPCV-D	-	-	+++	+++
PCV	+/-	-	-	+++

Table 1. Reactions of hybridization probes with 0.1μg IPCV and PCV RNA

serotype, probe 3 from ORF1 of RNA-1 reacted with RNA from all 3 IPCV serotypes and probe 4, which included the 3'-non-coding region, hybridized well with all the RNA types.

RT/PCR tests yielded clear bands of cDNA equivalent in size (for IPCV-H) to the predicted products. Thus the sequences shared between IPCV-H RNA-1 and PCV RNA-1 were also shared completely or nearly completely with RNA-1 of IPCV-L and IPCV-D.

Discussion

We have obtained a broad specificity hybridization probe which is being used currently in disease surveys in India. IPCV infects many hosts and multiplies well in graminaceous hosts

such as wheat (Delfosse *et al.*, 1995). Groundnut came from South America and presumably encountered the ancestors of the viruses inducing clump on arrival in the Old World. It is possible that in the weed flora there are viruses related to IPCV and PCV which do not infect groundnuts. The new probe might be expected to detect such viruses. The results also suggest that with any new virus isolate it will be possible by RT/PCR to identify how closely such a new virus is related to existing viruses.

From the sequences of the putative polymerases of IPCV and PCV, it would be reasonable to classify the viruses as strains. But other proteins encoded by RNA-1 suggest less close relatedness and those encoded by RNA-2 of IPCV and PCV (Naidu *et al.*, this volume) are even less alike. Practical considerations suggest that IPCV and PCV be considered as different viruses. Sequence comparisons also show that, as with the coat proteins (Wesley *et al.*, 1994), BSMV is relatively closely related to IPCV, and more so than are other furoviruses. Taken together, these data strongly suggest that the general class of viruses with rod-shaped particles and multipartite genomes require taxonomic re-alignment.

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