PROCEEDINGS OF THE SECOND SYMPOSIUM OF THE INTERNATIONAL WORKING GROUP ON PLANT VIRUSES WITH FUNGAL VECTORS

Editor: Chuji Hiruki

McGill University, Montreal, Canada
July 25-27, 1993

SEQUENCE STUDIES OF INDIAN PEANUT CLUMP FUROVIRUS RNA

J.S. Miller[1], V. Wesley[2], M.A. Mayo[1], D.V.R. Reddy[2], R.A. Naidu[2], M.K. Jana[3] & V.K. Pamiak[4].

(1) Scottish Crop Research Institute, Invergowrie, Dundee, UK, (2) ICRISAT, Patancheru, Hyderabad, A.P., India, (3) IIT, Kharagpur 721 302, West Bengal, India and (4) CCMB, Hyderabad 500 007, A.P. India.

Summary

Indian peanut clump virus (IPCV) has a bi-partite genome and is transmitted by *Polymyxa graminis*. it has been classified in the furovirus genus. Cloning and sequencing of cDNA made to RNA from IPCV particles has revealed that the 5'-proximal ORF on RNA-2 codes for the coat protein and that RNA-1 codes for a putative methyl transferase gene. The 3'-halves of each RNA have yet to be sequenced. IPCV coat protein is 61% identical to the coat protein of peanut clump virus from West Africa (PCV). This value, together with the lack of serological relatedness between them, confirms that IPCV and PCV should be considered as separate viruses. Comparisons among the coat proteins of a range of rod-shaped viruses showed that IPCV and PCV coat proteins resembled that of barley stripe mosaic hordeivirus more than those of other furoviruses.

Introduction

Indian peanut clump virus (IPCV) causes a serious disease in groundnut crops (Reddy *et al.*, 1988). It is soil-borne and is transmitted by the soil-inhabiting fungus *Polymyxa graminis* to groundnut and graminaceous hosts (Reddy *et al.*, 1988). IPCV can persist in soil for several years and is difficult to control; no resistance to IPCV has yet been found in groundnut germplasm (Reddy *et al.*, 1988).

The virus has rod-shaped particles of two lengths comprising a 24K coat protein and RNA molecules of either c.6kb (RNA-1) or c.4kb (RNA-2) (Reddy *et al.*, 1985). RNA-2 is the mRNA for the coat protein (Mayo and Reddy, 1985).

Although classified in the furovirus group because of its fungus transmission and rod-shaped particles (Brunt, 1991), IPCV is serologically unrelated to West African peanut clump virus (PCV) and other furoviruses. Isolates of IPCV from different places in India differ appreciably serologically (Reddy *et al.*, 1985; Nolt *et al.*, 1988).

In projects aimed at developing cloned probes to detect infection by IPCV and at isolating the coat protein genes for transformation work, we have sequenced about half of each RNA of IPCV. The results obtained so far have been used in sequence comparisons to infer taxonomic relationships of IPCV with other furoviruses.

Materials and Methods

cDNA synthesis and cloning

RNA was extracted from purified virus particles as described by Mayo and Reddy

(1985). cDNA was synthesised as described by Gubler and Hoffman (1983) using a commercial kit (Boehringer) and cloned in *Sma* I-cut pUC19. Clones specific to each IPCV RNA were identified by Northern blotting.

Nucleotide sequencing

Nucleotide sequences were determined by dideoxy chain termination (Sanger *et al.*, 1977). Template DNA was either single-stranded M13 DNA or double-stranded DNA from cDNA clones. Sequences were assembled using STADEN software (Staden, 1982) and analysed using the GCG package (Devereux *et al.*, 1984). Sequences were compared with those for PCV, barley stripe mosaic (BSMV), soil-borne wheat mosaic (SBWMV), beet necrotic yellow vein (BNYVV) and Nicotiana velutina mosaic viruses (NVMV).

Results

Nucleotide sequences

Two large contiguous sequences have been obtained. One corresponds to the 5'-half of RNA-2 which includes the coat protein gene and the other corresponds to the 5'-end of RNA-1 which includes part of what appears to be a methyl transferase gene.

In RNA-2 (Fig. 1), the 5'-most open reading frame (ORF) encodes a 24K protein which resembles the coat protein of PCV. When this gene was cloned into pET-15b, transformed *E. coli* expressed protein which reacted with antiserum to IPCV particles. The next ORF downstream is in the (-1) frame with respect to the coat protein gene and encodes a c. 39K protein (p39).

The sequence assembled for RNA-1 contains one ORF which extends 3' of the sequenced region. The encoded protein resembles a methyl transferase.

Sequence comparisons

Sequence comparisons among coat proteins of viruses with rod-shaped particles made using GAP revealed values greater than the non-specific value (c. 20%) only when IPCV was compared with PCV (61% match) and BSMV (37% match). Sequences were compared by multiple sequence alignments using CLUSTALV. The deduced relationships among the coat proteins (Fig. 2) show that BSMV is the virus most like IPCV and PCV and that coat proteins of other furoviruses are relatively distant from each other.

Fig. 3 shows the similarity between IPCV and BSMV coat proteins in a DOTPLOT. Two large areas of homology are clearly visible.

Discussion

Although IPCV and PCV are similar biologically, the value of a 61% match between the coat protein sequences confirms that the viruses are distinct and not one virus. In their coat protein sequences, IPCV and PCV more resemble BSMV than other furoviruses. Indeed there is little coat protein sequence similarity among furoviruses. This is the first report of strong coat protein sequence similarities between furoviruses and BSMV although some similarities have been found between non-structural proteins of BSMV and those of PCV (Manohar *et al.*, 1993), BNYVV (Bouzoubaa *et al.*, 1987) and SBWMV (Shirako and Wilson, 1993). It is possible that BSMV (which is not fungus-transmitted) and furoviruses had a common origin or have evolved by sharing genes by recombination in a common host.

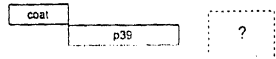

Fig. 1 Diagram of the deduced location of the coat protein and p39 genes in IPCV RNA-2. ? indicates the unsequenced region

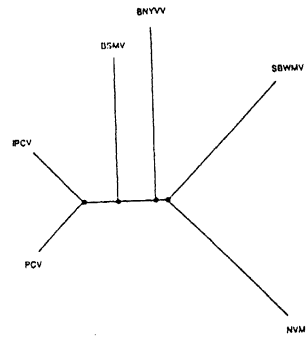

Fig. 2 Diagram of similarity relationships between furovirus coat proteins deduced from a CLUSTALV multiple alignment analysis

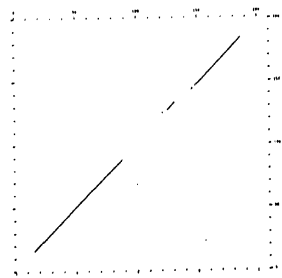

Fig. 3 DOTPLOT showing simirity between coat protein sequences of BSMV (vertical sequence) and IPCV (horizontal sequence)

References

Bouzoubaa, S., Quillet, L., Guilley, H., Jonard, G. & Richards, K. (1987). Nucleotide sequence of beet necrotic yellow vein virus RNA-1. *J. Gen. Virol.* 68:615-626.

Brunt, A.A. (1991). Furovirus group. In *Classisfication and Nomenclature of Viruses. 5th Report of the International Committee on Taxonomy of Viruses.* Edited by R.I.B. Francki, C.M. Fauquet, D.L.Knudson & F. Brown, p. 377-379. *Arch. Virol.* (Suppl. 2).

Devereux, J., Haeberli, P., & Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucl. Acids Res.* 12:387-395.

Gubler, U. & Hoffman, B.J. (1983). A simple and very efficient method for generating cDNA libraries. *Gene* 25:263-269.

Manohar, S.K., Guilley, H., Dollet, M., Richards, K. & Jonard, G. (1993). Nucleotide sequence and genetic organisation of peanut clump virus RNA-2 and partial characterisation of deleted forms. *Virology* 195:33-41.

Mayo, M.A. & Reddy , D.V.R. (1985). Translation products of RNA from Indian peanut clump virus. *J. Gen. Virol.* 66:1347-1351.

Nolt, B.L., Rajeshwari, R., Reddy, D.V.R., Bharathan, N. & Manohar, S.K. (1988). Indian peanut clump virus isolates: Host range, symptomatology, serological relationships, and some physical properties. *Phytopathology* 78:310-313.

Reddy, D.V.R., Robinson, D.J., Roberts, I.M. & Harrison, B.D. (1985). Genome properties and relationships of Indian peanut clump virus. *J. Gen. Virol.* 66:2011-2016.

Reddy, D.V.R., Nolt, B.L., Hobbs, H.A., Reddy, A.S., Rajeshwari, R., Rao, A.S., Reddy, D.D.R. & McDonald, D. (1988). Clump disease in India: isolates, host range, transmission and management. In *Viruses with Fungal Vectors.* Edited by J.I. Cooper and M.J.C. Asher, pp. 239-246.

Sanger, F., Nicklen, S. & Coulson, A.R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.(USA)* 74:5463-5467.

Shirako, Y. & Wilson, T.M.A. (1993). Complete nucleotide sequence and organisation of b bipartite genome of soil-borne wheat mosaic virus. *Virology* (In Press).

Staden, R. (1982). Automation of the computer handling of gel reading data produced by b shotgun method of DNA sequencing. *Nucl.Acids Res.* 10:4731-4751.

Acknowledgements

This work was supported by the Overseas Development Administration Plant Science Programme administered by the Centre for Arid Zone Studies, Bangor.