DEFECTIVE DNA ASSOCIATED WITH INDIAN CASSAVA-INFECTING GEMINIVIRUSES IN THE EXPERIMENTAL HOST *NICOTIANA BENTHAMIANA*

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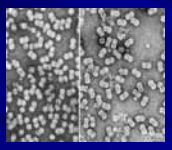
CASSAVA MOSAIC DISEASE



Cassava mosaic disease is one of the most important cause of loss in cassava production

Caused by different geminiviruses

Geminiviruses are plant viruses with twinned geminate particles enclosing single-stranded circular DNAs, which are usually of two types; DNA A and DNA B

CASSAVA MOSAIC DISEASE

- Caused by two bipartite geminiviruses *Indian cassava mosaic virus* (ICMV) and *Sri Lankan cassava mosaic virus* (SLCMV)

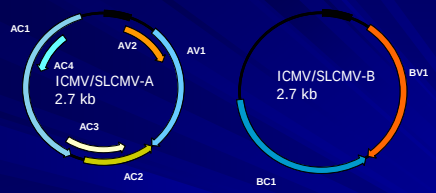
  Malathi and Srinivasan (1983) J. Root Crops 9: 69-73
  Hong et al. (1993) J. Gen. Virol. 74: 2437-2443
  Saunders et al. (2002) Virol. 293: 63-74
  Patil et al. (2005) Arch Virol. 150(2): 389-397
  Dutt et al. (2005) Arch. Virol. 150 (10); 2101-2108

- It was reported that SLCMV has a potential monopartite nature because DNA-A component could independently infect the experimental host *Nicotiana benthamiana*

  Saunders et al. (2002) Virol. 293: 63-74

- Several 2.7 kb full-length clones ICMV and SLCMV DNA-B components were cloned from CMD-affected cassava from various locations in Kerala in the last five years in our laboratory and their infectivity were tested on the experimental host *N. benthamiana*

ICMV and SLCMV have bipartite genomes



AC1: Replication associated
AC2: Transcriptional activator, Silencing suppressor
AC3: Replication enhancer
AC4: Silencing suppressor

AV1: Coat protein
AV2: Pre-coat protein
BC1: Movement protein
BV1: Nuclear shuttle protein

ICMV and SLCMV DNA-Bs cloned

Name	EMBL accession number	Size in kb	Site of collection
ICMV-[Ker3]	AJ575820	2715	Ernakulam
ICMV-[Ker6]	AJ512823	2758	Kozhikode
SLCMV-[Ker4]	AJ575821	2737	Thiruvananthapuram

All three DNA-Bs resembled previously reported ICMV and SLCMV DNAs from India

Symptoms in *Nicotiana benthamiana* following sap-inoculation from CMD-affected cassava leaves

Mock-inoculated

Inoculated with CMD-affected cassava leaf extract

Photograph taken two weeks post-inoculation

N. benthamiana is a good experimental system to study viral multiplication and spread

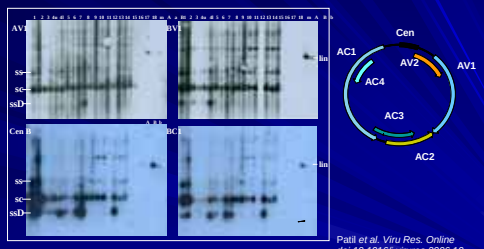
Symptoms of ICMV and SLCMV DNA inoculation on *Nicotiana benthamiana*

Symptoms at 4 weeks post-inoculation

Infectivities of the cloned DNA-Bs with standard DNA-As on *N. benthamiana*

DNA-A	DNA-B	No. infected/ no. inoculated
ICMV-[Mah2]	ICMV-[Ker3]	16/30
	SLCMV-[Ker4]	0/30
	-	0/25
SLCMV-[Col]	ICMV-[Ker3]	1/30
	SLCMV-[Ker4]	19/30
	-	0/25
ICMV-[Mah2] + SLCMV-[Col]	ICMV-[Ker3]	5/10
	SLCMV-[Ker4]	3/10
	ICMV-[Ker3] + SLCMV-[Ker4]	4/10
	-	0/10

Southern analysis of infected *N. benthamiana*



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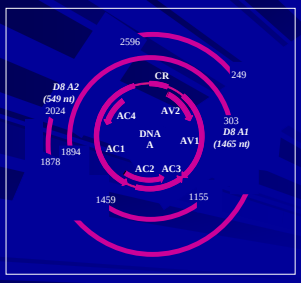
Defective DNA species, which were faster migrating on agarose gels were observed along with super-coiled and single-stranded forms of the viral DNA

Defective DNAs cloned from DNA-A



Using PCR, defective DNAs were cloned and sequenced

Those derived from DNA-A had suffered upto two deletions

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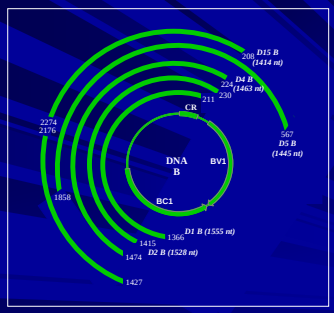
Defective DNAs cloned from DNA-B



Defective DNAs from DNA-B generally suffered one deletion and were missing *BV1* partially or completely

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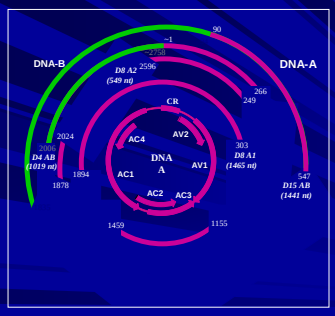
Recombinant defective DNAs



Recombinant defective DNAs were detected for the first time in geminiviruses

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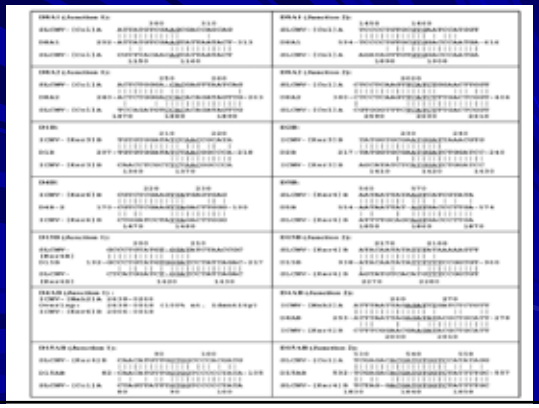
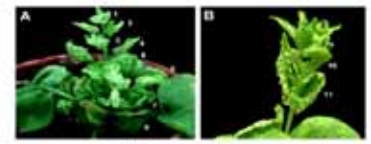
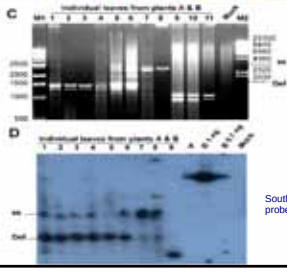
Defective DNA junctions were derived from repeats



Defective DNAs and symptom change



Few inoculated plants showed a change of symptoms from downward leaf curl to upward leaf roll from lower to upper leaves



PCR with CR as primers

Southern with DNA-B as probe

The upward rolling leaves contained more defective DNA-B and less DNA-B

It is possible that this imbalance led to the change in symptoms

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Conclusions

- Several full-length DNA-B molecules from ICMV and SLCMV representing CMD in Kerala have been cloned and analyzed
- Infected *N. benthamiana* plants showed high accumulation of defective DNA species
- The defective species were cloned and found to be derived from DNA-A, DNA-B and some were even recombinants, the first such report from geminiviruses
- Recombination junction points corresponded to repeat sequences of the parental molecules, pointing towards homologous recombination events as their cause
- Some recombinant defective DNAs caused a change in symptom of the host, which correlated with change in levels of defective and the parental DNA
- More studies are required to study similar events in the natural host cassava to reveal the dynamics of ICMV and SLCMV infection and evolution

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