The role of a geminiviral DNA β satellite in viral pathogenicity and movement

by

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to my father

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Abstract

Geminiviruses (family *Geminiviridae*) have circular single-stranded genomes encapsidated in twinned quasi-isometric particles and are responsible for major crop losses worldwide. The largest genus, *Begomovirus*, comprises viruses transmitted by the whitefly *Bemisia tabaci*. Most begomoviruses have bipartite genomes, termed DNA A and DNA B. The DNA A component encodes proteins required for viral DNA replication and encapsidation whereas the DNA B encodes two proteins that are essential for systemic movement. A small number of begomoviruses have a monopartite DNA genome that resembles the DNA A of bipartite begomoviruses. This DNA carries all gene functions for replication and pathogenesis. Specific small circular single-stranded DNA satellites containing a single open reading frame (ORF), termed DNA β, have recently been found in association with certain monopartite begomovirus infections. They comprise about 1350 nucleotides and require a helper begomovirus for replication and encapsidation. DNA β contributes to the production of symptoms and enhanced helper virus accumulation in certain hosts. This study examines the role of DNA β satellite in viral pathogenicity and movement in the host plant.

Infectivity analysis of *Tomato leaf curl virus* and DNA β having mutation in the C1 and V1 ORF indicated that the complementary-sense ORF, βC1, is responsible for inducing disease symptoms in *Nicotiana tabacum*. An ORF present on the plus strand, βV1, appeared to have no role in pathogenesis. Tobacco plants transformed with the βC1 ORF under the control of the C*auliflower mosaic virus* 35S promoter, or with a dimeric DNA β exhibited severe disease-like phenotypes, while plants transformed with a mutated version of βC1 appeared normal. Northern blot analysis of RNA from the transgenic plants using strand-specific probes identified a single complementary-sense

transcript. The transcript carried the full βC1 ORF encoding a 118 amino acids product. It mapped to the DNA β nucleotide (nt) position 186-563 and contained a polyadenylation signal 18 nt upstream of the stop codon. A TATA box was located 43 nt upstream of the start codon. These results indicate that βC1 protein is responsible for DNA β induced disease symptoms.

Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus in which both DNA A and DNA B are required for systemic infection. Inoculation of tomato plants with ToLCNDV DNA A alone induced local but not systemic infection whereas co-inoculation with DNA A and the DNA β resulted in systemic infection. The presence of both DNA A and the DNA β in systemically infected tissues and the absence of DNA B was confirmed by probe hybridization. DNA β containing a disrupted βC1 ORF did not mobilize the DNA A for systemic infection. Co-inoculation of plants with DNA A and a construct of βC1 ORF, under the control of the *Cauliflower mosaic virus* 35S promoter, resulted in the systemic movement of the DNA A. βC1 fused to GFP accumulated around and inside the nucleus, at the periphery of tobacco and onion epidermis cells and co-localized with the endoplasmic reticulum. This distribution would be consistent with βC1 mediating intra cellular transport from the nucleus to the plasma membrane. These results showed that the βC1 protein can replace the functions of DNA B in allowing the systemic movement of a bipartite geminivirus DNA A.

Declaration

This work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Muhammad Saeed

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Publications

Sections of this thesis have been published (see Appendix 1) or in preparation for publication, as follows.

Saeed, M., Behjatnia, S. A., Mansoor, S., Zafar, Y., Hasnain, S. & Rezaian, M. A. (2005). A single complementary-sense transcript of a geminiviral DNA beta satellite is determinant of pathogenicity. *Mol Plant Microbe Interact* **18**, 7-14.

Saeed, M. Randles, J. W. Zafar, Y. and Rezaian, .M. A. A monopartite begomovirus-associated DNA β satellite substitutes for the DNA B of a bipartite begomovirus to permit systemic infection (Manuscript in preparation for J. Gen. Virol.)

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Abbreviations

AbMV	*Abutilon mosaic virus*
ACMV	*African cassava mosaic virus*
AYVV	*Ageratum yellow vein virus*
AP	alkaline phosphatase
AYVV	*Ageratum yellow vein virus*
BCTV	*Beet curly top virus*
BDMV	*Bean dwarf mosaic virus*
BGMV	*Bean golden mosaic virus*
bp	base pair
BYVMV	*Bhendi yellow vein mosaic virus*
CaMV	*Cauliflower mosaic virus*
cDNA	complementary DNA
ChiLCuV	*Chili leaf curl virus*
CLCuAV	*Cotton leaf curl Alabad virus*
CLCuGV	*Cotton leaf curl Gezira virus*
CLCuMV	*Cotton leaf curl Multan virus*
CLCuRV	*Cotton leaf curl Rajasthan virus*
CP	coat protein
C	complementary-sense
CSR	complementary-strand replication
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
dpi	days post-inoculation
dsDNA	double-stranded DNA
dsRNA	double-stranded RNA

EDTA	ethylenediamine-*tetra*-acetic acid
ER	endoplasmic reticulum
EpYVV	*Eupatorium yellow vein virus*
g	gram(s)
g	relative centrifugal force
GFP	green fluorescent protein
GUS	ß-glucuronidase
h	hour(s)
HoLCrV	*Hollyhock leaf crumple virus*
HYVMV	*Honeysuckle yellow vein mosaic virus*
ICMV	*Indian cassava mosaic virus*
IR	intergenic region
kb	kilobase pairs
L	litre(s)
LB	Luria broth
M	molar
min	minute(s)
MOPS	3-N-Morpholinopropanesulfonic acid
MP	movement protein
mRNA	messenger RNA
miRNA	micro-RNA
MSV	*Maize streak virus*
MYVV	*Malvastrum yellow vein virus*
MYVYV	*Malvastrum yellow vein Yunnan virus*
NLS	nuclear localisation signal
NSP	nuclear shuttle protein

NTP	nucleoside triphosphate
nt	nucleotide
ORF	open reading frame
ori	origin of replication
OYVMV	*Okra yellow vein mosaic virus*
PaLCuV	*Papaya leaf curl virus*
PCR	polymerase chain reaction
Pd	Plasmodesmata
PM	Plasma membrane
PTGS	post-transcriptional gene silencing
RCR	rolling circle replication
RDR	recombination-dependent replication
REn	replication-enhancer protein (encoded by *AC3/C3*)
Rep	replication-associated protein
RF	replicative form
RNA	ribonucleic acid
rpm	revolutions per minute
rRNA	ribosomal RNA
s	second(s)
SDS	sodium dodecyl sulphate
SiYVV	*Sida yellow vein virus*
siRNA	small interfering RNA
SLCMV	*Sri Lankan cassava mosaic virus*
SqLCV	*Squash leaf curl virus*
SSC	standard sodium citrate
ssDNA	single-stranded DNA

ssRNA	single-stranded RNA
TbCSV	*Tobacco curly shoot virus*
TBE	tris-borate-EDTA
TbLCYV	*Tobacco leaf curl Yunnan virus*
TGMV	*Tomato golden mosaic virus*
TGS	transcriptional gene silencing
ToLCNDV	*Tomato leaf curl New Delhi virus*
ToLCJV	*Tomato leaf curl Java virus*
ToLCV	*Tomato leaf curl virus* (Australian isolate)
Tris	tris(hydroxymethyl)aminomethane
TPCTV	*Tomato pseudo-curly top virus*
TYLCV	*Tomato yellow leaf curl virus*
TYLCCNV	*Tomato yellow leaf curl China virus*
TYLCTHV	*Tomato yellow leaf curl Thailand virus*
V	volt(s)
VIGS	virus-induced gene silencing
V	Virion-sense
WT	wild-type
YFP	yellow fluorescent protein
ZiLCV	*Zinnia leaf curl virus*