A study of gene functions in Indian cassava mosaic virus

Abstract of the Ph.D. thesis submitted by

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Cassava (*Manihot esculenta*) is the staple or subsidiary food for about a fifth of the world's population, ranks second to sugarcane in carbohydrate content and is an important source of dietary calories. Cassava is infected by numerous geminiviruses causing cassava mosaic disease (CMD) that cause devastating losses to poor farmers in Africa and India. CMD causes severe mosaic, leaf distortion and stunted growth of plants, resulting in considerable reduction of yield. Geminiviruses are a large family of plant viruses with circular, single-stranded DNA (ssDNA) genomes packaged within geminate particles.

The causative agent of CMD in India is believed to be *Indian cassava mosaic virus* (ICMV). As a first step towards assessing the diversity of cassava infecting geminiviruses (CIGs) in India, we initiated the studies on molecular variants present in the field by using PCR-RFLP and multiplex-PCR analysis. These studies showed the prevalence and wide distribution of *Sri Lankan cassava mosaic virus* (SLCMV) in India and the presence of several novel types and recombinants. These observations were further confirmed by full length cloning and sequencing of a total of four full length viral genomic components.

The infectious nature of the cloned genomic components was demonstrated by inoculation on the experimental host *Nicotiana benthamiana.* The ability of the cloned DNAs to form viable pseudorecombinants between ICMV & SLCMV and multiple / mixed inoculation of ICMV & SLCMV DNAs were also tested to investigate the possible synergistic effects.

The generation of defective DNA (D-DNA) molecules, possibly associated with symptom production was demonstrated in *N. benthamiana*. Cloning and sequence

analysis of nine such D-DNAs ranging in size from 549 to 1555 nt revealed that they contained the *cis*-acting regions essential for DNA replication as well as portions of other viral genes for DNA-B derivatives. Moreover, evidence of deletion and, for the first time, recombination events between DNA-A and DNA-B components to produce D-DNA were obtained.

The nuclear-replicating geminiviruses require the active participation of the two movement-related proteins, NSP (Nuclear Shuttle Protein) and MP (Movement Protein), which act cooperatively to transport the viral genomes from its site of replication in the nucleus to the cell periphery. In order to gain a better insight into the nucleocytoplasmic transport of these viral genomes, the behavior of NSP gene of ICMV was analyzed by ectopic expression as GFP fusion in the epidermal cells of *N. benthamiana* leaves introduced by biolistic inoculation followed by the subcellular localization by both fluorescence and confocal microscopy. The GFP-NSP fusion protein showed nuclear localization and was seen to associate with the cell periphery in some cells as discontinuous and discrete bands. This association towards cell periphery might indicate its interaction with the cell wall and thus hinting towards the possible presence of PTS (plasmodesmal targeting signal). The nuclear localization of NSP was also confirmed in other hosts *N. tabacum* and cassava and a non-host, onion.

A number of deletion constructs of NSP fused with GFP were generated and their analysis revealed the presence of two bipartite NLSs in its N- terminal end (1-34 aa). Further, the N-terminal deletion analysis indicated the presence of a cell wall binding domain towards the C-terminal end. This is also supported by the concentration of glycosylation and myristoylation domains at the C-terminal end, known for their membrane binding.