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Title of thesis: **Structural Genomics, Synergy and Silencing of Cucumber mosaic virus and Tomato leaf curl virus**

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Abstract

To counteract the plant defense mechanism i.e. RNAi silencing, many plant viruses encode for specific silencing suppressors which allow the viruses to proliferate in their specific hosts. The extent to which the synergistic viral interactions occur in higher plants and role they play in mediating plant disease is not really clear at this point. To explore the molecular mechanism of viral synergism, an important threat of severe diseases and emergence of new viruses, the viral suppressors from two viruses belonging to different genera; ToLCV (Geminivirus) and CMV (Cucumovirus) were studied to explore their role in host gene regulation. To understand the molecular mechanism of infection, knowledge of viral genome sequences is must. For ToLCV genome sequence data from India is available, but in case of CMV no report of full length genome sequence from India was available. For development of viral resistance various approaches have been used which includes transgenic resistance. To explore possibility of transgenic resistance to the *Tomato leaf curl virus* through the principle of RNA interference (RNAi) present study was constituted. Among various protein coding ORFs of the ToLCV genome, coat protein (AV1) was considered to be suitable target for the RNA based silencing as it has been widely and successfully deployed for the resistance against the Begmovirus in general. Therefore, the present study was contemplated with objectives to: Characterizing genomic components of the viral genome of *Cucumber mosaic virus*, Synergism of viruses affecting tomato (ToLCV and CMV): In relation to expressions of suppressors and Expression of artificial microRNAs for silencing ToLCV infection.

The present study encompasses the following findings:

- Comparative sequence analysis reveals that the RNA1, RNA2 and RNA3 of CMV-New Delhi isolate from India shared 88.4-97.9%, 88.2-91.1% and 89.2-97.4% overall sequence identities at nucleotide level, respectively, with other CMV subgroup 1B strain.
- The phylogenetic relationship based on the complete nucleotide sequences of the 14 CMV strains revealed three distinct types of groups i.e. IA, IB and II, with CMV-ND falling in subgroup IB.
- CMV-ND, RNA1 and RNA3 showed maximum sequence identity at the nucleotide level with Taiwan isolate (97.9 and 97.4%, respectively), whereas RNA3 showed similar sequence identity with both Taiwan and Italy strains (91.1%).
- Phylogenetic analysis revealed that CMV-ND, CP gene is most conserved (93.4-99.5%) and 2b gene is least conserved (70.6-88.6%) among nucleotide sequences of CMV group I isolate.
- Recombination detection program proves that CMV-ND has naturally recombined with CMV subgroup IA and IB strains. The result indicates that RNA1 and 2 of CMV-ND are derived from subgroup IB, while RNA2 is derived from subgroup IA strain.

- Among 15 studied CMV genomes, most of the recombination sites are present in the 2a gene followed by 2b, while CP gene showed minimum number of recombination, suggesting that the RNA2 of CMV has played a vital role in the genome dynamics of CMV.
- Putative recombination sites detected *in silico* suggest that the whole CMV genome is prone to recombination. In addition, the molecular architecture of the CMV genome based on recombination provides a better understanding in terms of evolution, host specificity, and geographic distribution.
- Constitutive expression of 2b in healthy tobacco causes unregulated differentiation, chlorosis, apical apoptosis and underdeveloped leaf lamina while, its over-expression in CMV infected *N. tabacum* produced negligible phenotypic aberrations.
- 2b over-expression in CMV infected *N. tabacum* plants does not alter existing viral titre.
- 2b over-expression in ToLCNDV infected *N. tabacum* plants enhances the levels of ToLCNDV-AC4 viral titre.
- The results suggest that CMV-2b helps hetrologous ToLCNDV in its establishment in the host.
- Constitutive expression of AC4 in healthy tobacco causes major phenotypic aberrations like unregulated differentiation, severe stunting and malformed leaves in *N. tabacum*.
- The over-expression AC4 in ToLCNDV infected *N. tabacum* produced severe phenotypic aberrations most of the transformants did not survived after callusing.
- ToLCNDV-AC4 accumulated to significantly higher titres in double transformed (2b+AC4) and 2b transformed lines.
- The northern blotting and RT-qPCR results indicate enhanced accumulation of host miRNAs in plants expressing viral suppressors and also in virus infected plants.
- The 21-nt potent siRNAs targeting coat protein gene (AV1 ORF) of ToLCNDV was designed and incorporated into 273-nt miRNA backbone of *Arabidopsis thaliana* miR159a.
- The transgenic *N. tabacum* plants carrying artificial miRNAs can express mature 21-nt amiRNAs, without affecting the endogenous miR159a levels.
- The majority of the tomato and tobacco plants derived from transformation with amiRAV1 did not show viral symptoms as compared to control ToLCNDV infected tomato plants, suggesting viral silencing.
- The several checked amiRAV1 transformed tobacco and tomato plants showed 66% and 63% decrease in ToLCNDV symptoms respectively.
- The amiRNA strategy has an advantage in targeting various other virus variants, due to less extensive base pairing with target sequence than siRNAs. Therefore, amiRNA has possibility to engineer broad spectrum resistance to several other begomoviruses.