SUMMARY

Studies carried out in the thesis entitled, ‘Genome organization and mechanism of RNA silencing suppression of begomoviruses infecting some solanaceous crops’ focused on the scenario of emerging begomoviral diseases in sub-temperate areas of Kangra district, Himachal Pradesh, India and understanding viral suppression of RNA silencing. This is one of the pioneer studies related to begomoviral diseases in Himachal Pradesh. Begomoviruses are considered as one of the most destructive pathogens of economically very important crops. Begomoviruses were earlier thought to be prevalent mainly in tropical and subtropical parts of the world but now they are emerging as serious problems in more temperate areas of India probably due to global warming, green house cultivation, appearance and increase in the vector (whiteflies) population in these areas. Solanaceae is one of the plant families which are heavily attacked by the viruses. Its species occur in temperate as well as tropical regions. Diseases caused by begomoviruses have been among the major constraints to the cultivation of these crops worldwide.

During surveys (2006-2009) in District Kangra, Himachal Pradesh, many economically important crops and weeds were found to show yellowing, curling, vein clearing and mosaic kind of symptoms on the leaves associated with whiteflies, indicating begomovirus infection. Whiteflies in this area were initially observed mainly in greenhouses and polyhouses but now they easily flourish in open fields as well. Begomovirus symptoms in this area were first observed in earlier years of last decade. Virus diagnosis was performed using slot-blot hybridization and polymerase chain reaction techniques. Complete genomes of begomoviruses were amplified by the latest rolling circle amplification method, cloned and sequenced using primer walking.

A distinct begomovirus species was diagnosed to cause leaf yellowing and curling disease of tomato. Complete bipartite genome was molecularly characterized (accession numbers AM884015 and AM992534) and a name ‘tomato leaf curl Palampur virus’ (ToLCPMV) was proposed for this new species. No satellite molecule was detected from the symptomatic plants. Recombination analysis suggested that this new begomovirus could have emerged as a result of recombination between Tomato leaf curl New Delhi virus and Squash leaf curl China virus like ancestors. To prove Koch’s postulates, agro-
infectious clones of the virus consisting of 1.4 and 1.8 mer direct tandem repeats of DNA-A and DNA-B components respectively were constructed in a binary vector. These clones could produce typical symptoms in tomato and *N. benthamiana* plants. DNA-B was found to be essential for systemic infection and induction of leaf yellowing and downward curling symptoms. Elemental analysis using CHNS analyzer revealed that ToLCPMV infection reduces the quantity of essential elements (carbon, hydrogen, nitrogen and sulfur) in stem, leaves and roots of the infected tomato plants in comparison to the non-infected plants. ToLCPMV was later reported to cause up to 100% loss of cucurbit crops growing in greenhouses in Iran. ToLCPMV has also been reported to cause a severe disease in bitter gourd in Pakistan. ToLCPMV along with *Pepper leaf curl betasatellite* has recently been reported to naturally infect pumpkin in India. ToLCPMV has also been reported to cause severe yellow leaf curl disease on muskmelon in Pakistan. Till date, fifteen complete genome and some partial sequences of ToLCPMV infecting tomato, melon, cucumber, pumpkin and bitter gourd have been submitted to EMBL database from different parts of the world by various research groups. This shows that the virus has a wide host range and geographical distribution and it can emerge as one of the most serious begomovirus problems in future. Keeping in view the growing importance of this virus, exploration of the mechanism by which this virus overcomes the natural host-defense (RNA silencing) was framed as one of the thesis objectives.

Chilli leaf curl disease in this area was found to be caused by a distinct begomovirus species for which a name ‘chilli leaf curl Palampur virus’ (ChiLCPaV) was proposed. *Chilli leaf curl virus* was also detected in this region. ChiLCPaV has a monopartite genome associated with *Chilli leaf curl betasatellite*. Complete genome was sequenced (accession numbers FM877858 and FM877803) and the virus appeared to be a recombinant of *Tomato leaf curl Karnataka virus* and *Croton yellow vein mosaic virus*. Agro-infectious clones were constructed, encompassing 1.9 and 1.7 mer direct tandem repeats of the viral and betasatellite components, respectively. The clones were agro-inoculated to chilli and *N. benthamiana* plants. The viral clone caused stunting of chilli plants when inoculated alone and it produced typical upward leaf curl symptoms in combination with the betasatellite clone. However the virus could replicate systemically without the need of betasatellite.
**Summary**

*Tomato leaf curl New Delhi virus* was found to be associated with potato plants showing apical leaf curl symptoms in Himachal Pradesh. Complete genome of the isolate was molecularly characterized (accession numbers AM850115 and FN356024) and it had closest identity with other ToLCNDV isolates, reported from potato. This was the first report of a begomovirus infecting potato in Himachal Pradesh. Common bean is another very important crop grown in this region. A yellow mosaic disease on this crop was observed and the causal pathogen was identified as an isolate of *Mungbean yellow mosaic India virus*. Complete bipartite genome of the isolate was sequenced (accession numbers FN794200 and FR714861). No satellite component was associated with either ToLCNDV or MYMIV.

*Zinnia elegans* is a popular flowering plant grown in this area. A leaf curl disease in this plant was found to be associated with *Ageratum enation virus* and an alphasatellite (DNA1) component. Complete genome of this monopartite begomovirus (FN543099) and the DNA1 component (FN543100) was sequenced. The complete sequence of this isolate had $\leq 93\%$ identity with all other AEV sequences available in GenBank which suggested that it might be a distinct strain of this virus. Any betasatellite was not detected from the infected samples. This was the first report of any begomovirus infection in *Zinnia* spp. in India and AEV infection throughout the world. Also the association of a begomovirus with an alphasatellite component without any betasatellite was novel. The commonly occurring weeds in this region viz. *C. crepidioides* (Benth.) S. Moore and *A. conyzoides* L. showing vein yellowing symptoms were also screened for begomoviruses. It is important to investigate the emergence of new begomoviruses, especially in weeds that may act as their reservoirs during the non-cropping season. AEV along with an alphasatellite were found to be associated with both the weeds. Complete AEV and alphasatellite sequences from *C. crepidioides* (accession numbers FN794201 and FN794202) and ageratum (accessions numbers FN794198 and FN794199) had 99% identity with each other and were closely related to the zinnia isolates. This was the first report on any begomovirus infection in *C. crepidioides* in India and the first on AEV infecting *C. crepidioides* worldwide and *A. conyzoides* in India. Any other begomovirus was not detected from these weeds.
To understand the mechanism of RNA silencing suppression by ToLCPMV, all its genes were cloned under CaMV 35S promoter in pCambia-1302 vector for their transient expression. In Agrobacterium co-infiltration assay, agro-inoculation of individual gene constructs in combination with green fluorescent protein (GFP) expressing pCambia-1302 was performed on wild type N. tabacum cv. Xanthi leaves. The assay focuses on early events of RNA silencing and AC4 protein of ToLCPMV came out as a suppressor in this assay. In reversal of silencing assay individual gene constructs were agro-inoculated to leaves of GFP silent transgenic N. tabacum cv. Xanthi. In this assay, AV2 protein of ToLCPMV could reverse RNA silencing against GFP, suggesting that it might be involved in suppression of established RNA silencing. The results were confirmed by reverse transcription-polymerase chain reaction (RT-PCR) of GFP mRNA using specific primers and sequencing of the amplicons. To understand the sub-cellular localization, these two genes were fused to GFP gene at their 3’ ends in pCambia-1302 vector and the resulting constructs were biolistically bombarded to onion epidermis cells. Free GFP (pCambia-1302 alone) localized in nucleus as well as cytoplasm whereas tagged AC4:GFP protein localized at the cell periphery near plasma membrane, suggesting that AC4 is a membrane protein. AC4 might be suppressing RNA silencing by interfering with the spread of systemic RNA silencing signal across cells. In contrast, AV2:GFP fusion protein localized exclusively in the nucleus. Studying mode of action of these two suppressors is an important future area of research.

CONCLUSIONS:
1. Begomoviruses are intruding into sub-temperate areas of India which is a matter of serious concern to sustainable agriculture in these areas. A scenario of emerging begomovirus diseases in a sub-temperate region in India is presented in the present study.
2. RCA is a simple, reliable and robust method in geminivirus genomics.
3. Tomato leaf curling and yellowing disease in Himachal Pradesh is caused by a hitherto unknown distinct begomovirus species that has been named as ‘tomato leaf curl Palampur virus’. ToLCPMV is a bipartite begomovirus and DNA-B is essential for systemic infection and symptom development.
4. Chilli leaf curl disease in Himachal Pradesh is associated with a hitherto unknown distinct begomovirus species that has been named as ‘chilli leaf curl Palampur virus’ and ChiLCV. ChiLCPaV is a monopartite begomovirus that can replicate systemically without the need of any other component and produce stunting symptoms on chilli plants. However it produces typical leaf curl disease only in combination with a betasatellite.

5. Interspecies recombination underlies the evolution of the newly emerged begomoviruses. ToLCPMV might have emerged due to recombination between ToLCNDV and SLCCNV-like ancestors whereas ChiLCPaV might be a recombinant of ToLCKV and CYVMV.

6. Genome properties, phylogeny and recombination analysis of ToLCNDV associated with potato apical leaf curl disease, MYMIV associated with yellow mosaic disease of common beans, AEV and alphasatellite components associated with leaf curl disease of zinnia and vein clearing disease of C. crepidioides and A. conyzoides is presented in the study. Weeds might be acting as reservoirs of begomoviruses during the non-cropping season. This is first report on any begomovirus infecting Zinnia spp., C. crepidioides and AEV infecting A. conyzoides in India. These AEV isolates represent a tentatively new strain. This is also a first report on association of an alphasatellite with a begomovirus without a betasatellite component. This is one of the pioneer studies related to begomoviral diseases in Himachal Pradesh.

7. AC4 and AV2 proteins of ToLCPMV were identified as suppressors of RNA silencing. AC4 might be involved in early events whereas AV2 can reverse established RNA silencing and might be involved in later events of RNA silencing. ToLCPMV AC4 is a membrane protein and might be interfering with cell to cell spread of RNA silencing signal but in contrast AV2 localizes in the nucleus and might be involved in suppression of established RNA silencing. ToLCPMV has a wide natural host range and geographical distribution and can emerge as a more serious problem. This study on suppression mechanism of the virus will be very useful in overall management of the virus and identification of host factors involved in RNA silencing.