Diversity and Interactions of Begomoviruses and Their Associated DNA-Satellites

Muhammad Naeem Sattar
Faculty of Natural Resources and Agricultural Sciences
Department of Plant Biology and Forest Genetics
Uppsala

Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2012

Acta Universitatis Agriculturae Sueciae
2012:023

Cover: Top right, whitefly *Bemisia tabaci* (begomovirus vector); top left, electron micrograph of twinned icosahedrals; Middle, genome organization of begomoviruses and associated satellites; Begomovirus infected plants: Bottom right-left, *Abelmoschus esculentus* and *Gossypium hirsutum* (Pakistan), *Ageratum conyzoides* (India).

ISSN 1652-6880
ISBN 978-91-576-7659-7

Print: SLU Service/Repro, Uppsala 2012

Diversity and Interactions of Begomoviruses and Their Associated DNA-Satellites

Abstract

To study the diversity of begomovirus/DNA-satellite complexes, plants of one crop species (okra; *Abelmoschus esculentus*) and one weed species (*Ageratum conyzoides*), both with leaf curling symptoms, were analyzed. Sequence analyses of complete genome components revealed a unique begomovirus/DNA-satellite complex in both plants. Okra was found to harbor infection with viruses of three begomovirus species, including the new recombinant species Okra leaf curl Cameroon virus, as well as one betasatellite and two divergent alphasatellites. The analysis of *A. conyzoides* revealed infection with a previously un-described complex of a begomovirus, betasatellite and alphasatellite. The results suggest that the diversity of begomoviruses and their associated DNA-satellites in Africa is much higher than previously thought, and that it may be similar to the diversity in Asia. Begomoviruses and satellites from different geographic regions, such as Africa and Asia, are genetically different. To test the compatibility of begomoviruses and satellites from Asia, Africa and the Mediterranean Region, inoculation experiments were carried out in the model host *Nicotiana benthamiana* using agroinfectious constructs for different begomoviruses and satellites. It was found that the tested begomoviruses and satellites could functionally interact with non-cognate betasatellites and alphasatellites with varying efficiencies. The results showed that the functional requirements for interaction between begomovirus and satellite can be quite relaxed. Thus, it is quite possible that new begomovirus-satellite combinations with components from different geographic regions will be formed, which may overcome plant resistance and increase the host range of begomoviruses. Functional studies were carried out on the betasatellite-associated monopartite begomovirus *Cotton leaf curl Kokhran virus*. Mutation analyses of the coat protein (*CP*), *V2*, *C2* and *C4* genes showed that CP, V2 and *C2* are pathogenicity determinants and that they are involved in facilitating virus movement and maintenance of betasatellites. *C4* is responsible for symptom induction, but it was not specifically required to maintain betasatellites.

Keywords: Plant disease, mixed infection, begomovirus, DNA saterllites, interaction, trans-replication, mutation, *Nicotiana benthamiana.*

Author's address: Muhammad Naeem Sattar, Uppsala BioCenter, Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences, and Linnean Center for Plant Biology, P.O. Box 7080, 750 07 Uppsala, Sweden

E-mail: Naeem.Sattar@ slu.se; Naeem.Sattar1177@gmail.com

Dedication

To the glorious love of my father and my mother, which I could never return back.

"The ink of the scholar is more holy than the blood of the martyr".

"He who travels in the search of knowledge, to him God shows the way of Paradise".

(Muhammad S.A.W)

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Leke W.N., Brown J.K., Ligthart M.E., **Sattar N.**, Njualem D.K., Kvarnheden A. (2012). *Ageratum conyzoides*: A host to a unique begomovirus disease complex in Cameroon. *Virus Research* 163, 229-237.

II Leke W.N., **Sattar M.N.**, Ngane E.B., Ngeve J.M., Kvarnheden A., Brown J.K. Diverse helper begomoviruses and satellite DNAs infecting okra plants in Cameroon. Manuscript

III **Sattar M.N.**, Ligthart M.E., Kvarnheden A. Molecular comparisons and trans-replication studies of begomoviruses and DNA satellites causing leaf curl disease in Asia and Africa. *Manuscript*

IV Iqbal Z., **Sattar M.N.**, Kvarnheden A., Mansoor M., Briddon R.W. Effects of the mutation of selected genes of *Cotton leaf curl Kokhran virus* on infectivity, symptoms and the maintenance of Cotton leaf curl Multan betasatellite. *Manuscript*

The contribution of Muhammad Naeem Sattar to the papers included in this thesis was as follows:

I Participated in planning the experiments and carrying out the experimental work.

II Shared first author. Participated in planning the experiments and carrying out the experimental work. Took part in analysing the results and writing the manuscript.

III Planned the experiments and carried out most of the experimental work. Summarized the results and wrote the manuscript.

IV Participated in planning the experiments and carrying out the experimental work. Took part in analysing the results and writing the manuscript

Abbreviations

AA	Amino acid
ACMV	*African cassava mosaic virus*
ALCCMA	Ageratum leaf curl Cameroon alphasatellite
ALCCMB	Ageratum leaf curl Cameroon betasatellite
ALCCMV	*Ageratum leaf curl Cameroon virus*
ARF6	Auxin response Factor 6
A-Rich	Adenine rich
AYVB	Ageratum yellow vein betasatellite
AYVV	*Ageratum yellow vein virus*
BCTV	*Beet curly top virus*
BYVMV	*Bhindi yellow vein mosaic virus*
CaMV	*Cauliflower mosaic virus*
CLCuD	Cotton leaf curl disease
CLCuGeA	Cotton leaf curl Gezira alphasatellite
CLCuGeB	Cotton leaf curl Gezira betasatellite
CLCuKoV	*Cotton leaf curl Kokhran virus*
CLCuMB	Cotton leaf curl Multan betasatellite
CLCuMuV	*Cotton leaf curl Multan virus*
CMD	Cassava mosaic disease
CMGs	Cassava mosaic geminiviruses
COI	*Cytochrome oxidase-I* gene
CP	Coat protein
CR	Common region
DNA	Deoxyribonucleic acid
DPI	Days of post-inoculation
EACMV-UG	*East African cassava mosaic virus*-Uganda
ER	Endoplasmic reticulum
GDavSLA	*Gossypium davidsonii* symptomless alphasatellite
GMusSLA	*Gossypium mustilinum* symptomless alphasatellite

HR	Hypersensitive response
ICMV	*Indian cassava mosaic virus*
ICTV	International Committee on Taxonomy of Viruses
IR	Intergenic region
JA	Jasmonic Acid
LC	Leaf curl
miRNA	Micro RNA
MP	Movement protein
NES	Nuclear export signal
NJ	Neighbor joining
NSP	Nuclear shuttle protein
NW	New World
OLCCMV	*Okra leaf curl Cameroon virus*
OLCD	Okra leaf curl disease
OLCuBFA	Okra leaf curl Burkina Faso alphasatellite
ORF	Open reading frame
Ori	Origin of replication
OW	Old World
OYCrCMA	Okra yellow crinkle Cameroon alphasatellite
OYCrV	*Okra yellow crinkle virus*
OYVMV	*Okra yellow vein mosaic virus*
PepGMV	*Pepper golden mosaic virus*
PepLCLV	*Pepper leaf curl Lahore virus*
PHYVV	*Pepper huasteco yellow vein virus*
PreCP	Pre-coat protein
PTGS	Post transcriptional gene silencing
PYMPV	*Potato yellow mosaic Panama virus*
PYMV	*Potato yellow mosaic virus*
QTL	Quantitative trait loci
RCA	Rolling circle amplification
RCR	Rolling circle replication
RDR	Recombination-dependent replication
REn	Replication enhancer protein
Rep	Replication associated protein
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
RNAi	RNA interference
SCR	Satellite conserved region
siRNA	Small interfering RNA
SLCMV	*Srilankan cassava mosaic virus*

ssDNA	Single-stranded DNA
TbCSV	*Tobacco curly shoot virus*
TGS	Transcriptional gene silencing
ToLCCMA	Tomato leaf curl Cameroon alphasatellite
ToLCCMV	*Tomato leaf curl Cameroon virus*
ToLCGHV	*Tomato leaf curl Ghana virus*
ToLCKuV	*Tomato leaf curl Kumasi virus*
ToLCNGV	*Tomato leaf curl Nigeria virus*
ToLCNV	*Tomato leaf curl New Delhi virus*
ToLCTGV	*Tomato leaf curl Togo virus*
ToLCV	*Tomato leaf curl virus*
ToLCVV	*Tomato leaf curl Vietnam virus*
TrAP	Transcriptional activation protein
TYLCCNDB	Tomato yellow leaf curl China betasatellite
TYLCCNV	*Tomato yellow leaf curl China virus*
TYLCD	Tomato yellow leaf curl disease
TYLCSV	*Tomato yellow leaf curl Sardinia virus*
TYLCTHV	*Tomato yellow leaf curl Thailand virus*
TYLCV-IL	*Tomato yellow leaf curl virus*-Israel
TYLCV-OM	*Tomato yellow leaf curl virus*-Oman
TYLCVV	*Tomato yellow leaf curl Vietnam virus*
UV	Ultra violet
YV	Yellow vein
βC1	Beta C1 protein

1 Introduction

1.1 Viruses

Definitions can be devised for a virus species but the definition of a virus as an entity is not fully understood yet (Van Regenmortel, 2006). Generally speaking, a virus is an infectious and potentially pathogenic parasite in living organisms. A virus can be defined as "Small obligate intracellular parasites, which either contain an RNA or DNA genome surrounded by a protective, virus-coded protein coat", or "A nucleoproteic structure having a single type of nucleic acid, either DNA or RNA", or in a broader way, "Intracellular parasites with nucleic acid capable of directing their own replication that do not serve any essential function for their host, have an extra-chromosomal phase and are not cells" (Roossinck, 2011).

1.2 Classification of viruses

Classification of viruses is a constant work in progress done by the International Committee on Taxonomy of Viruses (ICTV) with the identification and addition of new viral species. Over the past two decades classification of viruses has gradually evolved to name and group viral entities in a more pertinent and universally accepted way. The current classification by ICTV classifies viruses into families (-viridae), genera (-virus) and species on the basis of: the nature of their genome, RNA or DNA; single or double-stranded genome and positive/negative sense nucleic acid. So far, ~2000 virus species have been approved, divided into 6 orders, 87 families and 349 genera (Brown *et al.*, 2012). It has been suggested that there is a minimum of 10-100 viruses for each host species of microorganism, plant and animal. More than 1,000 different viruses can infect humans (Norrby, 2008). In plants, viruses are casual agents of ~47% of all emerging diseases (Anderson *et al.*, 2004).

Currently, there are 20 families with plant-infecting viruses sub grouped into 90 genera, which comprise ~800 plant virus species (Brown *et al.*, 2012). Generally, plant viruses have isometric or helical symmetry and vary in size ranging between 17 and 2000 nm (Hull, 2002).

1.3 Geminiviruses

Geminiviruses belong to the family *Geminiviridae*, which is the second largest plant virus family. They infect a broad range of plants including both, monocots (monocotyledonous) and dicots (dicotyledonous) (Gutierrez, 2000). During the last two decades worldwide economic losses, due to infections of geminiviruses, are estimated to be US $5 billion for cotton in Pakistan during 1992-97 (Briddon & Markham, 2001), in Africa US $1.3-2.3 billion for cassava (Thresh & Cooter, 2005), in India US $300 million for grain legumes (Varma & Malathi, 2003) and US $140 million in Florida for tomato alone (Moffat, 1999).

1.4 Family *Geminiviridae*

In tropical and sub-tropical regions of the world the typical symptoms (Fig. 1) of geminivirus infections have been observed in plants since the nineteenth century (Wege *et al.*, 2000). It was in the 1970s when a distinct group of single-stranded DNA viruses was found associated with these symptoms (Harrison *et al.*, 1977). This new group was later named geminivirus (Goodman, 1977) and later in 1980s classified into a new plant virus family *Geminiviridae* (Rybicki, 1994). The name was derived from Gemini, the Zodiac sign symbolized by twins (Harrison *et al.*, 1977). Most plant viruses have a genome of single-stranded (ss) RNA, while some viruses have ssDNA (geminiviruses and nanoviruses) or double stranded (ds) DNA (caulimoviruses). Viruses in the family *Geminiviridae* are characterized structurally by twinned (geminate) quasi-icosahedral capsids and genetically by having one or two small circular, ssDNA molecules. Geminiviruses replicate through an intermediate dsDNA molecule in the nuclei of infected host plant cells and depend upon the host DNA replication machinery (Jeske, 2007). Geminiviruses are independently replicating plant viruses, with the smallest known genome of plant-infecting viruses, utilize bidirectional mode of transcription and overlapping genes for efficient coding of proteins (Rojas *et al.*, 2005).

1.5 Geminivirus taxonomy and nomenclature

There are certain rules which should be followed to classify viruses into species, strains and variants (Van Regenmortel, 2006). The number of characterized geminiviruses is growing rapidly with each passing day. *Geminiviridae* is the second largest family among plant viruses and geminivirus taxonomy and nomenclature is becoming complex due to the increasing number of viral genomic sequences deposited. The taxonomic list of geminiviruses is regularly updated (Brown *et al.*, 2012; Fauquet & Stanley, 2005; Fauquet *et al.*, 2003) but there is a need for a rational and comprehensive way to describe and classify the newly identified geminiviruses. Therefore, to make the taxonomic standards more clear and nomenclature guidelines more transparent, ICTV has proposed a recent set of demarcation criteria (Brown *et al.*, 2012) for classification and naming of geminiviruses. It has been proposed that if the pairwise nucleotide sequence identity of a newly isolated geminivirus sequence to the already reported geminiviral sequences, using Clustal V algorithm, is <89% (except mastreviruses, which has <75% cut-off limit), it would be accepted as a new species whereas, a sequence identity >89% would be classified as a member of the same species. By comparing the nucleotide sequence of a geminivirus isolate to all known strains and variants if the pairwise comparison analysis <93% it would be a member of new strain of that species and if it would be >94% it is a variant of that strain the same species.

To name a new virus the following nomenclature structure is usually followed:

Species name, strain descriptor (symptoms, host, location, and/or a letter A, B, C etc)
[Variant descriptor (country: location: [host]: year)**]**

Till now more than 200 official geminivirus species have been recognized that have been divided into four genera (*Topocuvirus*, *Curtovirus*, *Mastrevirus* and *Begomovirus*) based upon genome arrangement, insect vector and sequence identities (Brown *et al.*, 2012). Each genus is named after the type species, e.g. Begomovirus-*Bean golden mosaic virus* (now named *Bean golden yellow mosaic virus*; (Brown *et al.*, 2012)). The genus *Begomovirus* is comprised of 196 member species, representing the largest genus of family *Geminiviridae* (Brown *et al.*, 2012).

1.6 Genus *Begomovirus*

Viruses of the genus *Begomovirus* are transmitted by the ubiquitous whitefly *Bemisia tabaci* and are the most numerous and economically most destructive viruses among the geminiviruses. During the last 30 years, begomoviruses have emerged as important viral pathogens in food, fiber, and ornamental crops in largest part of the world. Begomoviruses have been sub-divided into two types with either a bipartite or monopartite genome (Brown *et al.*, 2012; Seal *et al.*, 2006).

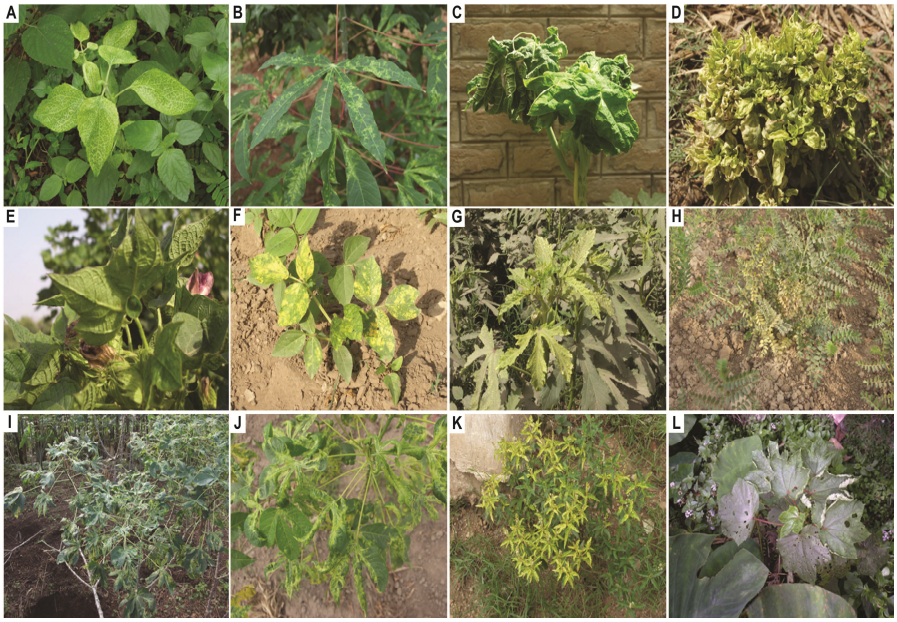

Figure 1. Symptoms caused by begomovirus infection of crops and weeds: *Ageratum Conyzoides* (A), Cassava (B) and Croton (C) in India, Pepper (D), Cotton (E), Mungbean (F), Okra (G) and Chickpea (H) in Pakistan, Cassava (I, J) and Papaya (K) in Uganda and Okra (L) in Cameroon.

1.6.1 Bipartite begomoviruses

Begomoviruses originating from the New World (NW) typically have genomes consisting of two separately encapsidated genomic components, known as DNA-A and DNA-B (Brown *et al.*, 2012) (Fig. 2). So far, no monopartite begomoviruses natives to the NW have been identified, although some monopartite begomoviruses have been introduced from the Old World (OW) (Zhang & Ling, 2011). In few cases among OW bipartite begomoviruses, DNA-A alone is sufficient for systemic infection and movement while the DNA-A component of NW begomoviruses is strictly dependent on the DNA-B component (Rojas *et al.*, 2005).

1.6.2 Monopartite begomoviruses

In the OW, although there is a small number of bipartite begomoviruses, the majority have genomes consisting of only a single genomic component, a homolog of DNA-A of bipartite viruses (Fig. 2). A small number of these begomoviruses in a sense are truly monopartite and capable of inducing disease alone in the field, such as *Tomato yellow leaf curl virus* (TYLCV) (Scholthof *et al.*, 2011).

1.6.3 Begomovirus-satellite complex

A majority of the monopartite begomoviruses associate with recently identified DNA satellites (betasatellites) and nanovirus-like DNA satellite molecules (alphasatellites) (Briddon & Stanley, 2006). This type of complexes mostly prevails in the OW including Asia and Africa. However, recently alphasatellites have been found associated with bipartite begomoviruses from the NW (Paprotka *et al.*, 2010; Romay *et al.*, 2010).

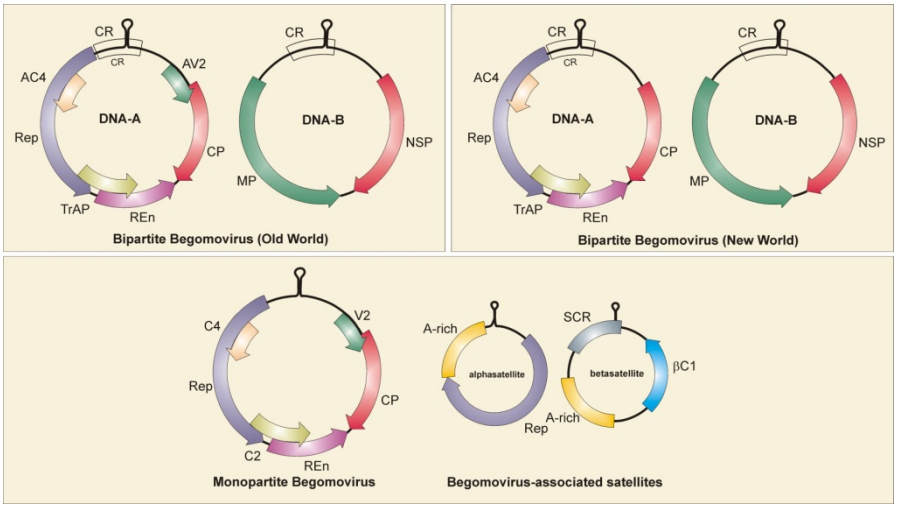

Figure 2. Genome organizations of begomoviruses and their associated DNA satellites.

Betasatellites

Failing attempts to reproduce yellow vein symptoms in *Ageratum conyzoides* (Fig. 1) by re-inoculation of *Ageratum yellow vein virus* (AYVV), suggested the presence of an additional factor responsible for symptom development (Saunders *et al.*, 2000). A number of recombinant molecules were then characterized from the infected host plant *A. conyzoides* that contain the begomovirus origin of replication (*ori*). After the discovery of recombinant molecules a novel ssDNA molecule, approximately half the size of helper

begomovirus, was isolated from *A. conyzoides* plants that reproduce typical yellow vein symptoms in the host plants (Saunders *et al.*, 2000). This molecule was named DNA-β and later named betasatellite. Although they have been recently identified (Briddon *et al.*, 2001; Saunders *et al.*, 2000), quite extensive work has been carried out to understand betasatellites. The betasatellites are very diverse in nature and are associated with helper begomoviruses. They are ssDNA molecules of approx. half the size of their helper begomoviruses (~1350nt) with no sequence homology with their helper viruses, other than the presence of a potential stem–loop structure containing the nonanucleotide sequence TAATATTAC (Briddon *et al.*, 2003) (Fig. 2). Betasatellites contain a region (~100 nt) known as the satellite conserved region (SCR), which is highly conserved between all betasatellites and contains the stem loop. In addition, the betasatellites contain an adenine rich region (A-rich) and a single ORF (the *βC1* gene) in the complementary orientation of the molecule.

Betasatellites depend entirely upon their helper begomoviruses for their replication, movement in plants, and transmission between plants, apparently by trans-encapsidation in the helper virus coat protein (CP). As will be discussed in the following section, rolling circle replication (RCR) of the genomes of geminiviruses is directed by the replication-associated protein (Rep), the only virus-encoded protein required for replication. Rep recognises small repeated sequences within the *ori* (known as iterons) that are distinct for each virus species. However, although betasatellites depend upon their helper virus for replication (thus presumably the helper virus-encoded Rep), they usually do not necessarily contain the iterons of their helper viruses. Initially, it was suggested that the SCR would be an important region for facilitating trans-replication of the betasatellite DNA by the helper virus, since the SCR includes an analogous position to the iteron sequence of the helper begomovirus, surrounding and upstream of the hairpin structure. However, this has been proven wrong (it will be discussed in detail in the section trans-replication). *In vitro* deletion analysis and availability of naturally occurring mutants suggest that, in fact, it is the sequence between the A-rich region and the SCR that may assist in Rep binding (Saunders *et al.*, 2008). This region is highly variable, contains sequences analogous to helper virus iterons and possibly provides an opportunity for betasatellites to adapt to helper begomoviruses.

All functions of betasatellites described so far, are mediated by the βC1 protein. The protein has multiple functions: pathogenicity determinant (determines the symptoms of the infection) (Fig. 3), suppressor of post-transcriptional gene silencing (PTGS) to overcome host plant defence, potential involvement in virus movement *in planta* (Saeed *et al.*, 2007), up-regulation of viral DNA levels *in planta* (Briddon *et al.*, 2001; Saunders *et al.*,

2000), potential binding of DNA/RNA (Cui *et al.*, 2005) as well as interaction with a variety of host factors (Eini *et al.*, 2009) and the CP of helper virus (Kumar *et al.*, 2006). Moreover, βC1 protein encoded by betasatellites also form multimeric complexes, which are essential for symptom induction in infected plants (Cheng *et al.*, 2011).

Alphasatellites

As was observed in the case of AYVV, the cloned genomic component of a begomovirus called Cotton leaf curl virus (later re-named *Cotton leaf curl Multan virus*, CLCuMuV) from Pakistan, was unable to induce typical leaf curl (LC) symptoms (Fig. 1) suggesting the presence of another factor (Briddon *et al.*, 2000). In an attempt to identify the causal agent for symptom induction in cotton, a nanovirus-like DNA satellite, named DNA-1 at that time, was found (Mansoor *et al.*, 1999). The component associated with begomovirus-betasatellite complexes was subsequently re-named alphasatellite. They are actually not true satellite molecules, because they are capable of self-replication and are thus designated as satellite-like molecules. These molecules are approx. half the size (~1400 nt) of the genomes of their helper begomoviruses, although somewhat larger in size than betasatellites (Fig. 2). They are highly conserved in their structure consisting of a single large gene, in the virion-sense orientation that encodes a Rep protein, an A-rich region and a predicted hairpin structure with a nona-nucleotide sequence (TAGTATT/AC) forming part of the loop (Briddon *et al.*, 2004; Mansoor *et al.*, 1999).

The initial analyses carried out for the first alphasatellite in Cotton leaf curl disease (CLCuD)-affected cotton plants, suggested that alphasatellites are not very diverse in nature. However, more recent studies conducted on CLCuD-affected plants of different *Gossypium* species from Pakistan (Nawaz-ul-Rehman *et al.*, 2010) and other recent studies, have shown that their diversity is much greater than previously assumed (Zulfiqar *et al.*, 2012; Mubin *et al.*, 2011). Although alphasatellites usually have been found in the OW associated with monopartite begomoviruses, and always in association with a betasatellite, recently alphasatellites have been characterized from the NW in association with bipartite begomoviruses and in the absence of betasatellites (Paprotka *et al.*, 2010; Romay *et al.*, 2010) and in the OW in association with monopartite begomoviruses in the absence of betasatellites (Leke *et al.*, 2011). However, these findings are not very odd, since an alphasatellite was shown experimentally to be maintained by a bipartite begomovirus from NW and even by a curtovirus, but not by a dicot-infecting mastrevirus (Lin *et al.*, 2003).

Further studies still need to be done to assess the benefits to a begomovirus-betasatellite complex offered by an alphasatellite. Previous experiments have

proven alphasatellites to cause a decrease in viral DNA levels in the plant, leading to the suggestion that when alphasatellites reduce virus titer, infected plants become less affected and may host begomovirus and onward transmission for a longer time period (Briddon *et al.*, 2004). More recently, Idris *et al.* (2011) have identified an unusual alphasatellite associated with TYLCV-OM (referred to as DNA 2) that besides ameliorating begomovirus symptoms significantly also reduces betasatellite DNA accumulation in plants. It is likely that the reduction in symptoms is due to the reduced betasatellite titer (thus less *βC1* expressed). However, the possible mechanism for reduction of the betasatellite remains unclear, since betasatellite replication depends upon helper begomovirus replication (Saunders *et al.*, 2008). Recently some symptomless alphasatellites have been demonstrated to encode Rep proteins acting as suppressors of gene silencing (Nawaz-ul-Rehman *et al.*, 2010).

1.7 Genome organization and potential functions of begomovirus encoded proteins

Apart from a short sequence of ~200 nt with high sequence identity that is referred to as "common region" (CR), and their size, the DNA-A and DNA-B components in bipartite begomoviruses are entirely different from each other.

The genomes of monopartite (and DNA-A components of bipartite) begomoviruses are typically ~2.8 kb in length and have genes in both orientations from a non-coding intergenic region (IR), which contains promoter elements and the *ori* of virion-strand DNA replication (Fig. 2). The virion-strand *ori* consists of a predicted hairpin structure containing a conserved (between geminiviruses) nonanucleotide (TAATATTAC) sequence in the loop and repeated upstream motifs known as "iterons". The DNA-A component of begomoviruses contains either five or six ORFs in both orientations that encode ~10 kDa proteins. These proteins are responsible for multiple functions: viral replication; host gene regulation and silencing suppression; virus assembly; and vector transmission. Although the genes are named on the basis of the functions they perform, yet the functions can differ within the genus *Begomovirus* (Bisaro, 2006; Vanitharani *et al.*, 2005).

The virion-sense strand of most begomoviruses encodes the following two proteins:

Coat protein (CP; V1): Coat protein, required for encapsidation, insect transmission and movement in plants (Sharma & Ikegami, 2009; Hanley-Bowdoin *et al.*, 1999). At some point during RCR, CP interferes with nicking

of DNA thus limiting the viral DNA copy number (Yadava *et al.*, 2010; Rojas *et al.*, 2005).

Pre-coat protein (Pre-CP; V2): A pathogenicity determinant, believed to be involved in virus movement in plants (Rojas *et al.*, 2005) and/or act as a suppressor of RNA silencing (Yadava *et al.*, 2010; Zrachya *et al.*, 2007). It takes part in perinuclear distribution of begomoviruses by association with endoplasmic reticulum (ER) and cytoplasmic strands (Sharma *et al.*, 2011). Begomoviruses native to the NW characteristically lack the *V2* gene.
The complementary-sense strand encodes four proteins:

Replication-associated protein (Rep; C1): The only virus-encoded gene product required for viral DNA replication. Rep is an RCR-initiator protein that recognises the reiterated motifs (iterons) and nicks within the nonanucleotide sequence to initiate replication (Nash *et al.*, 2011; Hanley-Bowdoin *et al.*, 2004). It also executes ATPase and helicase activities and binding of retinoblastoma-related proteins (Choudhury *et al.*, 2006; Pant *et al.*, 2001).

Transcriptional activator protein (C2; TrAP): Up-regulates the late (virion-sense) genes (for bipartite begomoviruses), serves as a suppressor of RNA silencing in bipartite (Yang *et al.*, 2007; Trinks *et al.*, 2005) and monopartite begomoviruses (Gopal *et al.*, 2007). It also overcomes virus induced hypersensitive cell death (Mubin *et al.*, 2010a; Hussain *et al.*, 2007).

Replication enhancer protein (REn; C3): REn is involved in establishing an environment conducive for virus replication (Pedersen & Hanley-Bowdoin, 1994). It also stimulates viral DNA replication (Pasumarthy *et al.*, 2011).

C4 protein: The function of the C4 protein still remains unclear but for some viruses it is a pathogenicity determinant and a suppressor of PTGS by binding of siRNAs (Pandey *et al.*, 2009; Vanitharani *et al.*, 2004).

As discussed earlier, the bipartite begomovirus genome consists of two components, i.e. DNA-A and DNA-B. In successful systemic infections both components are essential, where replication and transcription functions are provided by the DNA-A while DNA-B is responsible for inter- and intra-cellular movement of the virus. The DNA-B component contains two ORFs in opposite orientations encoding:

Nuclear shuttle protein (NSP; BV1): As evident from its name, NSP is responsible for transport of viral DNA from the nucleus into the cytoplasm (Malik *et al.*, 2005; Sanderfoot *et al.*, 1996).

Movement protein (MP; BC1): BC1 coordinates the movement of viral DNA across plasmodesmatal boundaries (Sanderfoot & Lazarowitz, 1996) and it is also responsible for viral pathogenic properties (Jeffrey *et al.*, 1996).

1.8 Vector transmission, begomovirus infection and replication mechanism

1.8.1 Vector transmission

As with all other geminiviruses, which require an insect vector to be transmitted to other plants, begomoviruses rely entirely on their arthropod vector the whitefly *B. tabaci* for their plant-plant transmission. There is vector specificity below the species level with several different biotypes of *B. tabaci*. Bedford *et al.* (1994) demonstrated vector specificity of 15 begomoviruses with the "B" biotype of *B. tabaci* as compared to other biotypes tested. This indicates that at least some begomoviruses have evolved to be transmitted (more efficiently) by specific biotypes. Whether the begomovirus vector *B. tabaci* is a species complex or a complex of different species, has for long been the subject of much debate. *B. tabaci* has long been considered as a species complex, but more recently on the basis of molecular characterization De Barro *et al.* (2011) have sub-divided *B. tabaci* into 11-well defined groups containing 24 species, although morphological they are indistinguishable from each other. On the other hand, depending upon different host plant preferences and virus transmission properties the indistinguishable *B. tabaci* populations are described as biotypes A to T (Seal *et al.*, 2006; De Barro *et al.*, 2005). The native whiteflies present in a region can affect the begomovirus prevalence in that particular region. This is because most biotypes can transmit a range of begomoviruses, but with differing efficiencies depending on virus species to be transmitted and the corresponding whitefly biotype (Maruthi *et al.*, 2002; Bedford *et al.*, 1994).

The vector *B. tabaci* (on the basis of *cytochrome oxidase I* gene) and the begomoviruses (on the basis of phylogenetic clustering of begomoviral CP and complete DNA sequences) show relationships according to geographical rather than host plant origin (De Barro *et al.*, 2005). However, due to free international trade and insect dispersal different biotypes of *B. tabaci* are no more confined to a specific geographic region. For example B biotype of *B. tabaci* has been introduced into America and the Mediterranean region from

the Middle East through trade of plants (Seal *et al.*, 2006). During a current epidemic of CLCuD in Pakistan, most part of the Sindh province has remained clear of the disease. Possibly, the *B. tabaci* biotype prevailing in this area is different from the rest of the country and thus the begomoviruses associated with the disease could not be transmitted (Ahmed *et al.*, 2010).

In the future, it will be interesting to investigate the basis for vector specificity with the proposed 24 "new" species of *B. tabaci* and to explore further the interaction between begomoviruses and their vector.

1.8.2 Begomovirus infection

The feeding of a viruliferous whitefly vector, *B. tabaci*, on the phloem cells of a suitable host plant leads to the beginning of the begomovirus infection cycle. As soon as the feeding starts, viral particles enter into the vascular system of the plant. From the cells in vascular system the viral particles are transmitted to the mesophyll cells. Once these viral particles are in the cells they become uncoated and viral DNA enters into the nucleus where viral DNA replication and transcription occur (Gafni & Epel, 2002). The replication of geminiviruses will be discussed in detail in the next section. For monopartite begomoviruses CP is responsible for the transfer of viral DNA into the host cell nucleus and later into the cytoplasm. Bipartite begomoviruses do not need CP for movement and they use NSP to act as a shuttle for virus movement from the nucleus into the cytoplasm (Malik *et al.*, 2005). In the nucleus the complementary strand is synthesized following primer synthesis to produce a dsDNA intermediate, which serves as a template for transcription of viral proteins (Settlage *et al.*, 2005). Once the dsDNA is formed, bi-directional transcription starts with the help of promoter sequences located in the IR. The viral transcripts are transported into the cytoplasm for translation (Hanley-Bowdoin *et al.*, 1999). The translated proteins enter into the nucleus to carry out replication, packaging and movement of viral DNA. The Rep protein of the begomovirus binds to the *ori* and starts RCR mode of replication. After accumulation of ssDNA CP switch RCR and shuttles ssDNA into the cytoplasm (long distance movement of begomovirus DNA will be discussed in detail in the preceding section). The CP starts packaging of the viral DNA to produce virions and the virus is either transported to the next cell through plasmodesmata or taken up by the whitefly to be transmitted to the next plant.

1.8.3 Replication mechanism

Since they lack their own DNA polymerase, begomoviruses, like all other geminiviruses, use the DNA replication machinery of their host to amplify their genomes in the nuclei of infected plant cells (Bisaro, 1996).

Begomoviruses replicate through a combination of RCR (Saunders *et al.*, 1991) and recombination-dependent replication (RDR) mechanism (Alberter *et al.*, 2005). After being translated the Rep protein is transported into the nucleus of the infected host cell to assist with viral DNA replication. Rep commences virus-specific recognition of its cognate *ori* (Hanley-Bowdoin *et al.*, 1999). The whole replication process can be divided into three phases: initiation - when Rep binds to the IR of the virus and produces a nick at the conserved nonamer sequence and produces a replication fork in coordination with host factors, i.e., RF-C, PCNA, RPA, RAD54, SCE1 and DNA polymerases etc. to start RCR (Kaliappan *et al.*, 2012; Sánchez-Durán *et al.*, 2011; Bagewadi *et al.*, 2004; Xie *et al.*, 1995); elongation - takes place at the 3'-OH end of the nick produced by Rep, which now acts as a helicase (Choudhury *et al.*, 2006); and finally termination - where Rep cuts and re-ligates the ssDNA to make it circular (Singh *et al.*, 2008). AC3/REn stimulates viral DNA replication depending upon the interaction of Rep with REn (Pasumarthy *et al.*, 2011). From sequence comparisons it has been shown that geminiviral Rep is a similar type of protein as those encoded by ssDNA plasmids for DNA binding (Koonin & Ilyina, 1992). Moreover, the binding of REn to the SINAC1 transcription factor also enhances viral DNA replication (Selth *et al.*, 2005).

The newly synthesized ssDNA follows one of the following passages: re-entry into the DNA replication cycle; packaging (encapsidation) and/or systemic transport to neighboring cells to spread the infection with the help of viral MPs.

1.8.4 Long distance movement within plants

When the viral DNA reaches an optimum level in the nucleus it is transported out of the plant cell nucleus to undergo systemic spread by crossing plasmodesmatal openings in the cell membrane. Bipartite begomoviruses are dependent upon DNA-B encoded NSP and MP for their movement in host plants (Gafni & Epel, 2002; Sanderfoot & Lazarowitz, 1996). The NSP supports viral DNA export from the nucleus into the cytoplasm from where MP transports viral DNA to neighboring cells via plasmodesmata (Lucas, 2006; Fontes *et al.*, 2004). It has been shown that βC1 of CLCuMB can substitute the movement function of DNA-B to facilitate movement of begomovirus from the nucleus to the cell periphery (Saeed *et al.*, 2007). Monopartite begomoviruses cross cell membranes with the help of interaction between CP and Pre-CP (Sharma *et al.*, 2011). The CP of monopartite begomoviruses localizes to the periphery of the nucleus and nucleolus, thus acting as a nuclear shuttle homologous to NSP of bipartite begomoviruses. Pre-CP localizes around the nucleus and at the cell periphery with the ER. Such a

localization pattern is similar to MP of bipartite begomoviruses, probably assigning movement function to these proteins (Priyadarshini *et al.*, 2011; Sharma *et al.*, 2011). The transport of viral ssDNA from the nucleus towards plasmodesmata is facilitated by a nuclear export signal (NES) on the CP C-terminus and NES on the Pre-CP N-terminus (Sharma *et al.*, 2011; Sharma & Ikegami, 2009).

1.9 Plant defense and begomoviral counter defense strategies

Plants usually offer resistance to geminiviruses through hypersensitive response (HR) (Mubin *et al.*, 2010a; Hussain *et al.*, 2005), mapping quantitative trait loci (QTLs) (Tomas *et al.*, 2011; Anbinder *et al.*, 2009) and DNA-methylation (Raja, 2010; Raja *et al.*, 2010) leading towards transcriptional gene silencing (TGS). RNA silencing (also known as RNA interference or RNAi) is a naturally occurring ubiquitous phenomenon in plants, animals and fungi that uses small interfering RNAs (siRNAs) or microRNAs (miRNAs), which recognize their target RNA in a sequence specific manner and degrade or suppress its translation (Obbard *et al.*, 2009; Matranga & Zamore, 2007). A functional RNAi pathway was adopted by eukaryotes more than one billon year ago (Matranga & Zamore, 2007; Cerutti & Casas-Mollano, 2006), where it plays an important role for controlling gene expression, development, genome stability, stress-induced responses and defense against molecular parasites such as transposons and viruses (MacLean *et al.*, 2010; Obbard *et al.*, 2009). The phenomenon of RNA silencing is important both for the host as well as for the pathogen. From host point of view it is a defense strategy providing innate immunity against transposons and viruses (Lodish *et al.*, 2008; Welker *et al.*, 2007), whereas it can be exploited by the pathogen to disrupt host cell functions, manipulating gene expression and counter host cell defense (Obbard *et al.*, 2009). The phenomenon has also been well described in plants (Ratcliff *et al.*, 1997). Plant viruses, whether RNA or DNA, elicit RNA silencing through the formation of double-stranded (ds) RNA, or RNA with ds regions, which is processed into small (21-24 nt) interfering siRNAs. siRNAs are in turn incorporated into an effector complex (the RNA induced silencing complex [RISC]) which leads to endonucleolytic cleavage of the target RNA or transcriptional repression, with specificity provided by the complementarity of the siRNA (Shimura & Pantaleo, 2011; Naqvi, 2010).

All viruses, which have been investigated, have evolved one or more suppressors to counter RNA silencing by the host plants (Bivalkar-Mehla *et al.*, 2011). The main targets of these silencing suppressors are either the RISC

components, ds or ss forms of siRNA and/or silencing signals to interfere with the host silencing machinery (Ding & Voinnet, 2007). Like other viruses, begomoviruses (either bipartite or monopartite) have also evolved suppressors of RNA silencing, e.g. C2/TrAP, C4 and V2/Pre-CP (Amin *et al.*, 2011a; Trinks *et al.*, 2005; Vanitharani *et al.*, 2004). However, many monopartite begomoviruses, associated with DNA-satellites, also have satellite encoded PTGS suppressor proteins. For example, Rep of two alphasatellites and βC1 of CLCuMB associated with begomoviruses causing CLCuD have been shown to exhibit strong silencing activity (Amin *et al.*, 2011a; Nawaz-ul-Rehman *et al.*, 2010). These virus encoded suppressors of RNA silencing have not been shown to affect Dicer-like proteins directly, but they interfere with different steps in the RNAi pathway (Glick *et al.*, 2007; Bisaro, 2006). The developmental anomalies and affected plant organs such as leaves, flowers and fruits, shown by symptomatic, virus-infected plants are often reconcilable with the virus-induced alterations of RNA silencing pathways (Shimura & Pantaleo, 2011). Although some plant virus silencing suppressors also interfere with the miRNA pathway of RNA silencing, it is considered as a side effect of interaction between viral suppressors and shared components of miRNA and antiviral siRNA pathways (Dunoyer *et al.*, 2004). It is thus clear (not just for geminiviruses) that induction of symptoms in plants is an ultimate result of interference with the miRNA pathway. Quite recently, Amin *et al.* (2011b) concluded that begomoviruses upregulate miR167, which is complementary to AUXIN RESPONSE FACTOR6 (AFR6). Transgenic plants overexpressing miR167 were dwarfed, expressed vein swelling and enations, typical features of begomovirus infection. The idea is further supported by another study on developmental abnormalities of plants caused by transient expression of begomovirus genes, which is linked directly with modified levels of developmental miRNAs (Amin *et al.*, 2011c).

1.10 Begomovirus-vector-host plant interactions

Begomoviruses not only interact with each other in terms of synergism and interference but also interact with their transmission vector and the host plant where they continue their life cycle.

1.10.1 Interaction with the vector

When an insect vector feeds on a virus infected plant a small subset of the virus population is acquired. Therefore, feeding of a single whitefly on multiple host plants adds different types of viruses to the pool, which is then transmitted to other host plants. Whether or not the ultimate consequence of this sequential

feeding of *B. tabaci* on the plants infected with different viruses will affect the transmission of the already acquired virus from the vector is debatable. Experiments showed no effect on the transmission of already fed TYLCV when the vector was sequentially fed on *Tomato yellow leaf curl Sardinia virus* (TYLCSV) (Czosnek *et al.*, 2002). It has been found that a single insect can harbor ~600 million virions (approximately 1 ng viral DNA) (Czosnek *et al.*, 2002). The insect vector can retain begomoviruses (such as TYLCV) during its complete life time, but the transmission efficiency could decline with time. Moreover, TYLCSV has been reported to be transmitted to the progeny of the vector through eggs and nymphs, though viral DNA lost its infection ability (Bosco *et al.*, 2004).

There exists a direct relationship between the spread of begomoviruses and the increased population densities and high fecundity of the B biotype of *B. tabaci*, not only the virus spread within crops but also from wild plants and weeds. Distinct *B. tabaci* biotypes and genotypes are linked with begomovirus epidemics such as those related to the introduction of the B-biotype into the Western Hemisphere (Morales & Anderson, 2001). Other examples are the spread of CLCuD in the Indo-Pak sub-continent, influenced by the Indian genotype of *B. tabaci* (Ahmed *et al.*, 2010), cassava mosaic disease epidemic in Africa (Legg & Fauquet, 2004; Maruthi *et al.*, 2002) and spread of begomovirus infection with the silverleaf whitefly or sweet potato whitefly *B. tabaci* (B biotype) in the United States (Perring *et al.*, 1993). The comparison of an invasive B-biotype *B. tabaci* in China with the native non-invasive ZHJ1 whitefly revealed that the B-biotype whitefly population displaced the native whitefly population when fed on plants infected with *Tomato yellow leaf curl China virus* (TYLCCNV) or *Tobacco curly shoot virus* (TbCSV) (Jiu *et al.*, 2007; Jiu *et al.*, 2006).

Specificity of the virus-vector relationship resides in the viral CP, the only viral-encoded protein required for whitefly-mediated transmission (Brown & Idris, 2005; Azzam *et al.*, 1994). In its role as a transmission determinant , the CP interacts with unidentified whitefly proteins at the gut and salivary gland membranes, to facilitate vector-mediated transmission (Brown, 2007). In the midgut of the whitefly endosymbiotic bacteria are present that produce the GroEL homologue necessary for the survival of begomoviruses (Morin *et al.*, 2000; Morin *et al.*, 1999). The GroEL protein of endosymbionts has been associated with the insects for the last 200 million years (Baumann *et al.*, 1993). However, quite recently Zhang *et al.* (2012) showed that Tomato yellow leaf curl China betasatellite (TYLCCNB) is responsible for initiating an interaction between begomovirus and *B. tabaci*. Where the βC1 protein acts as a suppressor of the jasmonic acid (JA) pathway and contributes to the

accumulation and enhances the performance of *B. tabaci* on plants. It is known that JA acid pathways in many plants lead to the plant defense against the insects (Pieterse & Dicke, 2007) and thus repression of JA pathway may increase the suitability of the insects.

After continent separation, the initial whitefly-begomovirus complex (es) has developed with time, due to many factors, into geographically separated and co-adapted virus-insect combinations (Bradeen *et al.*, 1997).

1.10.2 Interactions with the host plant

It is apparent that the genomes of begomoviruses show extreme plasticity leading to an ability to evolve very rapidly in response to changing cropping systems. A specific plant host in an area may, directly or indirectly, affect the particular vector population in that particular area. For example, the density of whitefly populations on smooth leaved cotton cultivars may differ from those with hairy leaves and ultimately it affects the selection of a particular whitefly biotype (Luqman, 2010). Two distinct whitefly populations for cassava and sweet potato have been reported from India, which ultimately altered the occurrence of host specific strains of viruses adapted with them (Lisha *et al.*, 2003). As different begomoviruses and/or strains differ in host range, the choice of crops may directly affect their prevalence in a particular region. For example in Spain a recombinant of TYLCV and TYLCSV was more pathogenic to a broader host range, as compared to its parents (Monci *et al.*, 2002). During a mixed infection, whether two viruses will interact with each other synergistically or antagonistically, depends upon the host plant in which they are interacting. In tobacco and *Nicotiana benthamiana*, *Pepper huasteco yellow vein virus* (PHYVV) and *Pepper golden mosaic virus* (PepGMV) showed synergism, but in pepper their interaction was antagonistic (Mendez-Lozano *et al.*, 2003). Whether or not a particular viral population will become pandemic, besides many other factors, is directly associated with host plant-virus-vector interaction (Colvin *et al.*, 2004).

It is now a well-established fact that the emergence of certain epidemics of geminiviruses is due to the introduction of new crops. The viruses may have been present already in the region, but when they came across to the new host plants they got adapted (Nawaz-ul-Rehman & Fauquet, 2009). Weeds and wild relative of crop plants are important as begomovirus sources. They yield new viruses and strains by acting as “melting pots” for recombination and assortment between begomoviruses (Azhar *et al.*, 2010; Mubin *et al.*, 2010b). The outcome of the interaction between begomoviruses and host plants also depends on the ability of the virus to counter-act the plant defense e.g PTGS (Moissiard & Voinnet, 2004; Vanitharani *et al.*, 2004). Cultivation of plants

with begomovirus resistance is a desirable option for many farmers (Thresh & Cooter, 2005) but widespread use of resistant cultivars can also change the diversity of the begomoviruses. Type of resistance in the host plant may determine the selection pressure on the virus. Use of resistant cultivars exerts a high selection pressure on the virus and ultimately more aggressive virus type would be selected. For example in Uganda, the most virulent strains of *East African cassava mosaic virus* (EACMV-UG) were most commonly found in the resistant cultivars as compared to the susceptible ones. On the other hand, virus tolerant cultivars provide a virus source for infecting susceptible cultivars and it may lead to the selection of viral strains with high accumulation rate but decreased virulence (Van Den Bosch *et al.*, 2006).

1.11 Cross continent genetic diversity of begomoviruses

Due to global human activity, transport of plant hosts and vectors by global trade and new agricultural practices coupled with climate change, the diversity of viruses and their strains is increasing. Begomoviruses are quite diverse in nature. During the past couple of decades they have increased in terms of their number, prevalence and distribution throughout the world with the identification and characterization of more and more begomoviruses (Navas-Castillo *et al.*, 2011; Fargette *et al.*, 2006). All begomoviruses, irrespective of their geographical distribution in OW or NW, co-evolved from a common origin. This is quite evident from the similar genomic organization of begomoviruses except for the absence of the *V2* gene in begomoviruses from the NW. The genotypic variation among plant viruses arise due to the occurrence of errors during their replication, which later get maintained in the following progenitors. Geminiviruses, like RNA viruses, have high substitution rates, similar to animal viruses (Duffy & Holmes, 2009; Duffy & Holmes, 2008). But ultimately recombination plays the major role in diversification of viruses (it will be discussed in detail in the next sections).

On the basis of their geographical distribution, begomoviruses have been classified as Africa-Mediterranean, Indian, Asian, and legume-infecting begomoviruses from the OW, while the NW begomoviruses have been classified into Latin American and Meso American groups. A seventh group of Sweet potato-infecting viruses (sweepoviruses) is found in both the OW and NW (Fauquet & Stanley, 2003). The geographical barriers are the main cause of seven major sub-divisions of begomoviruses, which are further sub-divided into 34 sub-populations (Prasanna *et al.*, 2010). Despite the close spatial association and evidence of relatively frequent recombination between

members of Asian and African begomovirus populations these sub-populations have still remained genetically quite distinct (Prasanna *et al.*, 2010).

1.11.1 Diversification of begomoviruses in Asia and Africa

The modern concept of diversification suggested eight different centers of plant origin (Harlan, 1971). Particular plant families are more diverse in their centers of origin as compared to the periphery of the centers. Geminivirus centers of diversification were identified from the phylogenetic tree constructed from all available sequences (Fauquet *et al.*, 2008) as the areas from where the maximum number of geminiviruses has been identified. On the basis of all available data about geminiviruses Nawaz-ul-Rehman and Fauquet (2009) identified eight distinct geographical centers of diversification for geminiviruses. These are Australia, Japan, South China, Indian sub-continent, Sub-Saharan Africa, Mediterranean-European region, South America and Central America. On the basis of all available data so far, most of the genetic diversity of geminiviruses is in the Chinese and Indian centers, which host ~46% of geminivirus species, 94% of betasatellites and 98% of alphasatellite species. Thus most of the known viral and DNA-satellite diversity is in the Indian sub-continent and China. The centers of crop and geminivirus diversity differ only for the Australian, African and South American centers. For example, cassava and cotton, which are native to NW as their center of origin are not infected by the same number of species of begomoviruses as compared to their introduced geographical areas (Fauquet *et al.*, 2008; Idris & Brown, 2004). Such situation points towards invasion of introduced crops in an area by the local begomoviruses (Nawaz-ul-Rehman & Fauquet, 2009).

In Indo-Pak sub-continent the begomoviruses are highly diverse. A small number of them are bipartite such as *Indian cassava mosaic virus* (ICMV) and *Tomato leaf curl New Delhi virus* (ToLCNDV) (Padidam *et al.*, 1995; Hong *et al.*, 1993), but most are monopartite associated with satellites, e.g. CLCuD-associated begomoviruses (Kirthi *et al.*, 2004), *Pepper leaf curl Lahore virus* (PepLCLV) and *Bhindi yellow vein mosaic virus* (BYVMV) (Fauquet *et al.*, 2008). Most of the satellites are prevailing in OW, particularly in the Indo-Pak subcontinent (Nawaz-ul-Rehman & Fauquet, 2009). Betasatellites are maintained by many begomoviruses, but many begomoviruses are associated with a particular type of betasatellites in the fields. For example, CLCuD is associated with more than seven begomoviruses, but only a single species of betasatellite, CLCuMB.

Africa also has tropical or sub-tropical climatic conditions more or less similar to the Indian sub-continent and the begomovirus diversity is high. Both types of begomoviruses, i.e., bipartite and monopartite begomovirus have been

reported from Africa. These begomoviruses infect a range of host plants including cassava, tomato, tobacco, okra and beans (Leke *et al.*, 2011; Tiendrébéogo *et al.*, 2010; Tiendrébéogo *et al.*, 2009; Zhou *et al.*, 2008; Lefeuvre *et al.*, 2007). Moreover, begomoviruses from weeds also act as a potential source for recombination and offer "new blood" for the begomovirus diversity (Leke *et al.*, 2012). In Africa, there are only two betasatellite species reported so far, Cotton leaf curl Gezira betasatellite (CLCuGeB) (Idris *et al.*, 2005) and Ageratum leaf curl Cameroon betasatellite (ALCCMB) (Leke *et al.*, 2012), but there are various alphasatellites reported (Leke *et al.*, 2012; Leke *et al.*, 2011; Tiendrébéogo *et al.*, 2010; Kon *et al.*, 2009).

The following factors are known to govern the genetic diversity of begomoviruses:

1.11.2 Mutation

Mutation occurs during several different biological processes like replication slippage or it can be induced by UV-light, chemical treatments etc. Incorporation of a non-complementary nucleotide during duplication of DNA or RNA gives rise to point mutations, which alters the genetic information. Usually RNA viruses have high mutation rates due to their dependence on error-prone RNA dependent RNA polymerase for their replication (Jenkins *et al.*, 2002). The mutation rate of RNA viruses was thought to be higher than DNA viruses (Domingo & Holland, 1997), but the mutation rate of TYLCV, TYLCCNV and EACMV was found to be almost similar ($\sim 10^{-4}$ substitutions/site/year) to RNA viruses (Fondong & Chen, 2011; Duffy & Holmes, 2009; Duffy & Holmes, 2008; Ge *et al.*, 2007), despite the fact that DNA viruses a use DNA polymerase from the host plant with high proofreading. However, it seems that DNA viruses do not utilize the DNA repair machinery from the host plants to repair any mismatches, which ultimately get maintained in the progeny. It seems clear that geminiviruses have a population structure very similar to RNA viruses (Ge *et al.*, 2007). The mutation rate of begomoviruses is considered to be affected by type of virus, host plant, age of the host plant and inoculum homogeneity. Geminiviruses especially begomoviruses and mastreviruses, have high mutation rate in wild as well as cultivated hosts (van der Walt *et al.*, 2008; Arguello-Astorga *et al.*, 2007; Sanz *et al.*, 1999). In spite of the low genetic diversity among CLCuMuV isolates, the mutation frequency of three ORFs (*CP*, *C1*, and *C4*) exceeded that of many plant and animal viruses (Sanz *et al.*, 1999).

Thus, occurrence of frequent mutations in DNA viruses suggests that genetic mutations in viruses are not an outcome of polymerase fidelity as in case of RNA viruses, but several other causes, i.e. genomic architecture,

replication speed, and host plant (Duffy & Holmes, 2008). Although recombination is the main source of emergence of new species and strains (García-Andrés *et al.*, 2007b; Lefeuvre *et al.*, 2007), but accumulation of point mutations also contributes to the viral diversity in a plant virus family (van der Walt *et al.*, 2008).

1.11.3 Recombination

Recombination is a process in which segmental exchange occurs between two strands of DNA or RNA during replication. From sequence comparison of different genera of family the *Geminiviridae* it is evident that topocuviruses have evolved as a result of recombination between a mastrevirus and a begomovirus (Rojas *et al.*, 2005; Briddon *et al.*, 1996). Interspecific homologous recombination has been described as an active driving source for begomovirus genetic diversity and contributes towards their evolution (Sanz *et al.*, 1999). Many epidemics of begomoviruses are reported as directly linked to recombinant viruses, for example, cassava mosaic disease (CMD) in Africa (Bull *et al.*, 2006; Pita *et al.*, 2001), tomato yellow leaf curl disease (TYLCD) in Spain, Africa and Mediterranean basin (García-Andrés *et al.*, 2007a; Idris & Brown, 2005), and CLCuD in Indo-Pak sub-continent and some parts of Africa (Idris *et al.*, 2005; Lisha *et al.*, 2003; Zhou *et al.*, 1998). CMD is an important disease prevailing in many cassava growing countries of the world characterized by at least eight begomoviruses (Brown *et al.*, 2012; Legg *et al.*, 2011; Fauquet *et al.*, 2008). The establishment of a recombinant virus depends on its selective advantage compared to its parental populations (Schnippenkoetter *et al.*, 2001). Like for CMD, recombination is a key player in spreading CLCuD in Pakistan and neighboring areas, as new variants are continuously being added into the CLCuD complex. CLCuD involves several different begomovirus variants, which have evolved through recombination between *Okra yellow vein mosaic virus* (OYVMV) and other begomoviruses (Kirthi *et al.*, 2004; Zhou *et al.*, 1998). In this region new disease complexes are emerging in the cultivated crops tomato, tobacco, pepper and papaya, and these diseases are very likely to be associated with new begomovirus recombinants (Venkataravanappa *et al.*, 2011; Zaffalon *et al.*, 2011).

Emergence of new begomoviruses depends upon how frequently begomoviruses recombine and ultimately begomoviruses are likely to acquire satellite molecules to further complicate the story (Zaffalon *et al.*, 2011; Nawaz-ul-Rehman & Fauquet, 2009). Begomoviruses not only recombine with each other but they can also experience recombination with satellite molecules or DNA-B (Venkataravanappa *et al.*, 2011; Nawaz-ul-Rehman & Fauquet, 2009; Tao & Zhou, 2008; Urbino *et al.*, 2003). In geminiviruses *ori* is

considered to be the most favorable site for recombination (Lefeuvre *et al.*, 2009; Tao & Zhou, 2008; Zhou *et al.*, 1998). Natural exchange of CRs occur either between DNA-A of two begomoviruses, such as *Potato yellow mosaic virus* (PYMV) and *Potato yellow mosaic Panama virus* (PYMPV) (Urbino *et al.*, 2003), or between DNA-A of *Srilankan cassava mosaic virus* (SLCMV) and DNA-B of ICMV (Saunders *et al.*, 2002). Recombination is not restricted to DNA-A only. There are several examples available where recombination has been identified between a begomovirus and a betasatellite, e.g., betasatellite recombinants with AYVV and TYLCCNV have been characterized (Tao & Zhou, 2008; Saunders *et al.*, 2001). Recombination events, where SCR has been exchanged with the CR of a begomovirus (Briddon *et al.*, 2001; Saunders *et al.*, 2001), and with a functional *βC1* might result in the formation of new betasatellites with higher virulence as compared to the existing ones (Nawaz-ul-Rehman & Fauquet, 2009). Moreover, presence of defective forms of betasatellites with deleted *βC1* or SCR replaced by CR of a begomovirus might explain the evolutionary link between begomoviruses and betasatellites.

As discussed above, the CR of begomoviruses is a hot-spot for recombination. It has been found that begomoviruses can exchange their CR with the alphasatellites as well. Quite recently (**Paper-I**), two types of defective begomovirus molecules have been characterized, which consisted of the complete IR and partial *CP*, *V2* and *Rep* from helper begomovirus *Ageratum leaf curl Cameroon virus* (ALCCMV) and partial *Rep* of Ageratum leaf curl Cameroon alphasatellite (ALCCMA). The second defective molecule was a recombinant, which had experienced a recombination event between ALCCMA, ALCCMV and ToLCTGV. Previously, similar types of defective forms of begomoviruses have also been characterized from *A. conyzoides* and cotton (Zaffalon *et al.*, 2011; Saunders & Stanley, 1999; Stanley *et al.*, 1997). The presence of such recombinant/defective forms of begomoviruses may play a role in their diversification and adaptation to the new hosts and environment. This might be one of the reasons that geminiviruses have such diverse nature with more than 200 species described so far.

1.12 Trans-replication

As discussed earlier, the DNA-B of bipartite begomoviruses and betasatellites depend entirely upon their helper begomovirus. In case of bipartite begomoviruses, the interaction between Rep and iteron sequences is essential for trans-replication of both DNA-A and DNA-B (Gladfelter *et al.*, 1997). Even though betasatellites have no sequence homology with their helper begomoviruses except the nonanucleotide sequence, yet, they are trans-

replicated by their helper virus (Mansoor *et al.*, 2003a; Saunders *et al.*, 2002). For instance, CLCuMB can be trans-replicated by AYVV, but CLCuMuV (a cognate helper virus for CLCuMB) cannot support Ageratum yellow vein betasatellite (AYVB) in trans-replication (Saunders *et al.*, 2008). Moreover, betasatellites can also be trans-replicated by geographically divergent and biologically diverse begomoviruses **(Paper-III)**. For example, ACMV and *Beet curly top virus* (BCTV - a curtovirus), which belong to different genera and differ extensively in sequence from each other, trans-replicated a defective molecule associated with *Tomato leaf curl virus* (ToLCV) lacking any ORF (Dry *et al.*, 1997). Similarly, TYLCV can trans-replicate distinct betasatellites from Japan and the Philippines (Ito *et al.*, 2009). Betasatellites can substitute for DNA B of bipartite begomoviruses and in turn they are trans-replicated by the begomoviruses as it has been shown for ToLCNV and CLCuMB (Saeed *et al.*, 2007) and AYVB trans-replication by cassava mosaic geminiviruses (CMGs) (Patil & Fauquet, 2010). Despite the fact that betasatellites have a relaxed requirement for high affinity Rep-binding motifs (Saunders *et al.*, 2008; Mansoor *et al.*, 2003a) **(Paper-III)**, geminiviruses still show differential interaction with betasatellites in trans-replication experiments (Patil & Fauquet, 2010). It seems that the βC1 protein of betasatellites can serve the functions of movement proteins (BC1 and BV1) for bipartite begomoviruses. Even if they are trans-replicated successfully by a non-cognate helper virus, two betasatellites, if inoculated together with a helper virus, may accumulate to a lower level in the plants and compete with each other (Tahir *et al.*, 2011; Qing & Zhou, 2009; Paper-III).

It has also been reported that monopartite begomoviruses can support the replication of a heterologous DNA-B component. For example, DNA-B of *Tomato yellow leaf curl Thiland virus* (TYLCTHV) can be trans-replicated by *Tomato leaf curl Vietnam virus* (ToLCVV) and *Tomato yellow leaf curl Vietnam virus* (TYLCVV) (Blawid *et al.*, 2008). Thus due to relaxed requirement of Rep-binding motif for betasatellites and interaction between a monopartite begomovirus and DNA-B of a bipartite begomovirus, it cannot be ruled out that *in vivo* an interaction between helper virus and satellite exists for Rep binding other than the Rep-binding motif (Lin *et al.*, 2003).

Thus the ability of begomoviruses to trans-replicate DNA-satellites provides an insight into the ability of begomoviruses and their associated satellites to undergo diversification and adaptation to the environment.

2 Aims of the study

The main focus of this thesis is to reveal the diversity of complexes with monopartite begomoviruses and DNA-satellites infecting crop plants and weeds by analyzing the components of tow complexes from Cameroon: one in the crop plant okra and another in the weed *Ageratum conyzoides*. The prevalence and genetic diversity of begomoviruses in these areas is discussed. Moreover, the interactions between begomoviruses and satellites from Asia and Africa are also addressed, which will enhance our knowledge about the adaptability and evolution of new begomovirus complexes

The specific objectives were:

1. Molecular and biological characterization of begomoviruses and satellites from Cameroon that infect Ageratum and okra:

- To fulfill Koch's postulates and carry out sequence analysis of the components associated with disease.
- To be used in compatibility studies of Asian and African begomoviruses and their associated satellites.

2. Co-inoculation of begomoviruses and their associated satellites to assess the compatibility and trans-replication capability of begomoviruses and satellites from different geographic regions.

3. Effects of the mutation of selected genes of *Cotton leaf curl Kokhran virus* on infectivity and symptoms and the maintenance of Cotton leaf curl Multan betasatellite.

3 Results and discussion

3.1 Characterization of begomoviruses and satellites from Cameroon that infect Ageratum and okra

3.1.1 Identification and characterization of a begomovirus and satellites infecting *Ageratum conyzoides* in Cameroon.

A. conyzoides is a common and widespread weed (Billy goat weed; family *Asteraceae*) throughout tropical and sub-tropical regions worldwide. In Southeast Asia, plants of *A. conyzoides* with yellow vein (YV) symptoms have been found to be infected by a monopartite begomovirus, *Ageratum yellow vein virus* (AYVV), associated with Ageratum yellow vein betasatellite (AYVB) and one or several alphasatellites (Huang & Zhou, 2006; Bull *et al.*, 2004; Saunders *et al.*, 2004; Saunders *et al.*, 2001; Stanley *et al.*, 1997). In China and Indonesia, both YV and leaf curl (LC) are common disease symptoms observed in *A. conyzoides*, associated with additional bipartite and/or monopartite begomoviruses with DNA satellites (Xie *et al.*, 2010; Sakata *et al.*, 2008; Kon *et al.*, 2007; Huang & Zhou, 2006). As other wild plant species (Jones, 2009), *A. conyzoides* acts as a reservoir for many economically important begomoviral disease complexes (Kon *et al.*, 2006; Sukamto *et al.*, 2005; Mansoor *et al.*, 2003b). In Cameroon, *A. conyzoides* is frequently infested by *B. tabaci* and exhibits characteristic LC begomoviral symptoms but without any YV. In Africa, only an alphasatellite has been reported infecting *A. conyzoides* from Kenya, but no begomovirus/betasatellite was then characterized (Briddon *et al.*, 2004). Therefore, the main objective of our study was to identify and characterize, if present, any begomovirus-satellite complex from this weed plant. Three leaf samples of *A. conyzoides* from Cameroon, collected on FTA classic cards, were tested by PCR using universal degenerate begomovirus primers designed to amplify the core or middle region of the *CP* gene (Wyatt & Brown, 1996) for the presence of begomovirus. The

initial sequencing data revealed that the *CP* sequences had 90% nucleotide identity to *Tomato leaf curl Cameroon virus* (ToLCCMV). Based on the cloned *CP* sequences, two abutting primer pairs were designed to amplify full-length begomoviral genomes from the sample AGL14. Four clones (AGFG14, AGFG23, AGFG24 and pBAL) were selected for full-length sequencing in both directions. Sequence analysis revealed that the total genome lengths of the begomovirus isolates were 2790-2794 nt and they had all characteristics of begomoviruses from the OW. The four genomic sequences were 97.2-99.7% identical to each other, which means that they are isolates of the same begomovirus species. When compared to the available viral genomes in GenBank, they showed highest nucleotide sequence identity (84.3-88.5%) to a group of begomoviruses from West Africa infecting tomato (Leke *et al.*, 2011; Osei *et al.*, 2008). Thus, following the ICTV guidelines for species demarcation (<89%) (Fauquet *et al.*, 2008), all of the sequences are from members of a new tentative species of begomovirus, for which the name *Ageratum leaf curl Cameroon virus* (ALCCMV) was proposed. According to a phylogenetic analysis, ALCCMV grouped in a clade with tomato-infecting begomoviruses from West Africa (100% bootstrap), which was further divided into two subclades: i) ALCCMV, ToLCCMV and *Tomato leaf curl Nigeria virus* (ToLCNGV); ii) *Tomato leaf curl Ghana virus* (ToLCGHV), *Tomato leaf curl Kumasi virus* (ToLCKuV) and *Tomato leaf curl Togo virus* (ToLCTGV). A recombination event was identified in ALCCMV-[CM:Lio1:AGFG14:09] and ALCCMV-[CM:Lio4:pBAL:09], on N-terminus of V1 at nt coordinates 732-803 having ALCCMV and ToLCNGV as major and minor parents, respectively. Thus these begomoviruses in West Africa infect a common host or at least in part.

The rolling circle amplification (RCA) product was linearized with *BamHI* to obtain betasatellite-like DNAs (~1.3 kb) and full-length satellite molecules were amplified by PCR with new primers designed using sequencing data. From sequencing results only one type of betasatellite was detected from all three plants analyzed. Like all other betasatellites known so far, the betasatellite detected from *A. conyzoides* plants contained an A-rich region (58%), a single ORF (*βC1*) in the complementary orientation and a SCR with a stem loop (TAATATTAC). All nine sequences analyzed were genetically variable and shared 70.3-99.5% nucleotide identity. These sequences could be divided into five different genotypes: SatB33, SatB35, SatB6/SatB14, AMBF, ALBF/AL2B1/AGLI4B2/AGLI4B7, with sequence identity within each genotype 93.5-99.5% and 70.3-84.1% sequence identity between genotypes. No recombination was detected. All betasatellites had highest nucleotide sequence identity (37.1%) with CLCuGeB from Sudan. The phylogenetic

analysis grouped all nine betasatellites into a distinct clade (100% bootstrap) separate from all known betasatellites from Asia and Africa. Following the suggested species demarcation value for betasatellites (<78%) (Briddon *et al.*, 2008), the identified betasatellites were suggested to be members of the new species Ageratum leaf curl Cameroon betasatellite (ALCCMB).

The RCA product was used to amplify alphasatellite-like molecules and after sequencing two distinct alphasatellite genotypes were obtained that shared only 51-57% nucleotide sequence identity to each other. The SatA-1 clones resembled typical alphasatellites having an A-rich region, a single ORF in virion orientation to govern self-replication (Rep) and a stem loop structure resembling nanovirus nonanucleotide sequence (TAGTATTAC). They showed highest nucleotide sequence identity (79.4-80.4%) to an isolate of Tomato leaf curl Cameroon alphasatellite (ToLCCMA) reported from Cameroon (Leke *et al.*, 2011). Following the suggested demarcation threshold (<83%) for alphasatellite species (Mubin *et al.*, 2009), SatA-1 was named Ageratum leaf curl Cameroon alphasatellite (ALCCMA). On the other hand, the SatA-2 sequences were similar in size to SatA-1 (1383-1413 nt), but contained the nonanucleotide sequence of the helper begomovirus. Sat-2 was further sub-divided into SatA-2a and SatA-2b. The SatA-2a clones were likely recombinant defective begomovirus molecules between ALCCMV (sharing IR, partial *CP*, *V2* and *Rep*) and ALCCMA (partial *Rep*). The SatA-2b clones structurally resembled SatA-2a, but were recombinants between ALCCMV and an uncharacterized begomovirus. Nucleotides 387-1045 were 100% identical to ALCCMA, nt 1-384 were 95% identical to ALCCMV and nt 1031-1413 shared a sequence identity of 93% with ToLCTGV, which suggests that SatA-2b emerged as a result of recombination between ALCCMA and a second uncharacterized begomovirus.

In Africa, besides the discovery of an alphasatellite from *A. conyzoides* in Kenya (Briddon *et al.*, 2004), knowledge about weed hosts harboring begomoviral complex with associated DNA satellites has been lacking. With this study it has been shown for the first time that *A. conyzoides* hosts a begomovirus complex including associated betasatellites and alphasatellites in Africa. ALCCMV, ALCCMB and ALCCMA are quite divergent from the begomoviruses and satellites found to infect *A. conyzoides* in Asia. The identification of a second betasatellite ALCCMB in Africa (the first one is CLCuGeB) points towards a growing complexity of betasatellites in Africa. Moreover, recombinant defective begomovirus/satellite molecules were reported for the first time from Africa. Thus, the discovery of such a divergent viral complex with associated satellites and its effect on wild and cultivated crop species represents an exciting new challenge.

3.1.2 Molecular and biological characterization of helper begomoviruses and associated satellites in okra from Cameroon

Okra (*Abelmoschus esculentus*) is an economically very important crop throughout Africa. Okra hosts many pests and diseases and among them, okra leaf curl disease (OLCD) is the most damaging disease in many parts of West Africa (Swanson & Harrison, 1993). Symptoms of OLCD are represented by leaf curling and distortion, a green/yellow foliar mosaic, stunted growth and ultimately reduction in yield.

Leaf samples were collected from fifteen plants at three different study sites in Southwestern Cameroon **(Paper-II)**. Full-length viral genomes were amplified and cloned from four positive okra plants (Lik1, Njo5, Mue5 and Mue1) and as four clones (Lik11, GR9, GR17 and GR23) from Lik1 were sequenced, three clones (Njo52, TOC10, TOC15) from Njo5, two clones (Mue5 and BCG1) from Mue5 and one clone from Mue1. The size and genomic organization of all isolates resembled mono-partite begomoviruses from OW, having an IR with a stem loop and nonanucleotide sequence, two ORFs in virion sense and four ORFs in complementary sense orientation. Sequence comparisons showed that, Njo52, TOC15, GR9 and Mue1 were 92.7-93.7% identical to four isolates of the previously characterized begomovirus *Okra yellow crinkle virus* (OYCrV) from Mali (Kon *et al.*, 2009; Shih *et al.*, 2009) and 97.5-98.3% to each other. Thus, they were identified as members of the new strain OYCrV-Cameroon: OYCrV-CM[CM:Njo5:Njo52:07], OYCrV-CM[CM:Mue1:07], OYCrV-CM[CM:Njo5:TOC15:07], and OYCrV-CM[CM:Lik1:GR9:07]. The nucleotide sequences of Mue5 and BCG1 were 99.5% similar to each other whereas they shared highest nucleotide sequence identity at 89.6-95.8% with CLCuGeV and Hollyhock leaf curl virus (HoLCuV) (Kon *et al.*, 2009; Idris *et al.*, 2005; Idris & Brown, 2002). Hence they were suggested to be members of a new strain of CLCuGeV from Cameroon: CLCuGeV-CM[CM:Mue5:07] CLCuGeV-CM[CM:Mue5:BCG1:07]. These results showed that these are not recently introduced begomoviruses in this region and that they are rather indigenous strains. The clones Lik11, GR17 and GR23 from okra plant Lik1 and TOC10 from Njo5 shared highest sequence identity (85.5-87.8%) with three isolates of CLCuGeV from Egypt and Sudan and were 95-99% identical to each other. Thus following the ICTV-begomovirus demarcation and nomenclature, Lik11, GR17, GR23 and TOC10 were identified as members of a new begomovirus species for which the name Okra leaf curl Cameroon virus (OLCuCMV) is proposed: OLCuCMV-[CM:Lik1:Lik11:07], OLCuCMV-[CM:Lik1:GR17:07], OLCuCMV-[CM:Lik1:GR23:07] and OLCuCMV-[CM:Njo5:TOC10:07]. These ten okra-infecting begomovirus clones were

grouped into the three different clades: i) the CLCuGeV clade (99% bootstrap), ii) OLCuCMV clade and, iii) OYCrV clade (100% bootstrap).

The recombination analysis revealed that for all four isolates of OLCuCMV, the fragment containing *V1* and *V2* was >91% identical with the homologous two ORFs in OYCrV, whereas *C1*, *C2*, *C3* and *C4* shared >91% nt identity with their CLCuGeV homolog. Recombination analysis program RDP3 identified HoLCrV-[EG:Cai1:01] as major parent and CLCuGeV-CM as the minor parent in the recombination event. Recombinations in the *V1* & *V2* region of the genome, as has been reported earlier for begomoviruses of the Easter Hemisphere (Prasanna & Rai, 2007; Fauquet *et al.*, 2005), may confer a selective advantage for encapsidation and/or vector transmission of these begomoviruses.

The predicted Rep binding site with a directly repeated (GGTACTCA) and inverted repeat (TGAGTACC) sequences was identified for Lik11, GR17, GR23 and TOC10. The predicted Rep binding site was identified as the directly repeated sequences GGGGT for Njo52, TOC15 and GR9, GGTGT for Mue1 and similarly, the inverted repeat sequences were identified as ACCC. For Mue5 and BCG1, the predicted Rep binding site was identified as the directly repeated sequence GGGTCTCA and the inverted repeat sequence TGAGACCC. However, experimental evidence is required to confirm the functionality of these motifs in begomovirus replication.

The sequencing data revealed that each of the okra plant infected with OYCrV-CM, CLCuGeV-CM or OLCuCMV-CM was containing molecules of a single betasatellite species. The okra-infecting molecule contained all the features of a betasatellite having an A-rich region, an ORF (*βC1*) in complementary orientation and an SCR containing stem loop. All four betasatellites were 98-99% identical to each other and shared highest nt sequence identity (93.3%) with isolates of CLCuGeB from Burkina Faso. The association of a single betasatellite with three helper begomoviruses from Cameroon, as has already been reported for CLCuGeB from Burkina Faso (Kon *et al.*, 2009) revealed that it is promiscuous among its helper begomoviruses. The ability of betasatellites to associate with helper begomovirus of different species represents an evolutionary strategy used by betasatellite for their survival and adaptation. Such a strategy by, if contributed towards begomovirus-betasatellite evolution, further complicates the situation. The betasatellite neighbor joining (NJ) tree yielded two well supported betasatellite clades, i) African clade containing three betasatellites from Africa and ii) Asian/Middle East clade of betasatellites. The African CLCuGeB clade was further resolved into three sub-clades, a) the BF/CM/ML/NG clade, b) the Egypt clade and c) Sudan clade. The respective Rep-binding motif for helper

begomoviruses was not found from CLCuGeB-[CM], suggesting that there is not any universal requirement of this motif.

All four okra samples were positive for the presence of alphasatellites and alphasatellites from the plants Lik1 and Njo5 were sequenced. The alphasatellite sequences represented two distinct genotypes (Alpha-1 & Alpha-2), which were present in both okra plants, but with a maximum 37.9% nt identity to each other. The two Alpha-1 isolates shared 98.5% nucleotide sequence identity, and showed the highest nt identity, at 96.4 and 97.3%, respectively, with Cotton leaf curl Gezira alphasatellite (CLCuGeA) recently identified in Burkina Faso (Tiendrébéogo *et al.*, 2010). They have all common features of alphasatellites. The three analyzed Alpha-2 satellites (one sequence from Lik1 and two sequences from Njo5) shared 94.4-99.2% nt identity, and showed highest nt identity, at 94.1-95.2% with isolates of Okra leaf curl Burkina Faso alphasatellite (OLCuBFA) from Burkina Faso (Tiendrébéogo *et al.*, 2010). With other alphasatellites they shared highest nt identity (52.7%) with an isolate of *Gossypium mustilinum* symptomless alphasatellite (GMusSLA) from Pakistan. The phylogenetic analysis placed Alpha-1 and Alpha-2 into two well supported alphasatellite clades, i) old alphasatellite clade, containing Alpha-1 and alphasatellites from Asia and Africa (80% bootstrap), and ii) novel alphasatellite clade, containing Alpha-2, GMusSLA and *Gossypium davidsonii* symptomless alphasatellite (GDavSLA) from Pakistan, and OLCBFA from Burkina Faso (Tiendrébéogo *et al.*, 2010). This clade was further resolved into (i) the Pakistani sub-clade (99% bootstrap) and (ii) the West African sub-clade, including isolates of Alpha-2 from Cameroon and isolates of OLCuBFA from Burkina Faso (99% bootstrap).

This relationship of Alpha-1 with other alphasatellites from Burkina Faso supports a strong phylogeographic association between them. Based on the >83% species demarcation value for alphasatellites (Mubin *et al.*, 2009) the Alpha-1 isolates were designated CLCuGeA-[CM:Lik1:d2:07] and CLCuGeA-[CM:Njo5:d2:07], whereas, the alpha-2 isolates were designated Okra yellow crinkle Cameroon alphasatellite (OYCrCMA-[CM:Lik1:sp3:07], OYCrCMA-[CM:Njo5:sp3:07] and OYCrCMA-[CM:Njo5:OY3:07]). OYCrCMA and its closest relatives represent novel alphasatellites that are quite divergent from all other know alphasatellites. Although both Alpha-1 and Alpha-2 were present within the same plant, yet they shared only 37.9% nt identity with each other. Moreover, the placement of Alpha-2 into a separate clade with unique and highly divergent alphasatellites is a surprising outcome of the phylogenetic analysis.

Plants of *N. benthamiana* were inoculated with the total RCA product from the infected okra plants Lik1, Njo5 and Mu5. All five plants inoculated with

RCA from Lik1 exhibited very strong begomoviral symptoms at 21 days post inoculation (dpi). In contrast, plants inoculated with RCA from Mu5 and Njo5 exhibited mild curling (Mue5) or symptomless infection. Sequencing results confirmed the presence of mixed infection of OLCuCMV, OYCrV, CLCuGeB and OYCrCMA in plants inoculated with RCA product from Lik1.

The *N. benthamiana* plants inoculated with RCA product from Njo5 were confirmed for the presence of OYCrV and/or OLCuCMV, CLCuGeB and OYCrCMA. The plants inoculated with Mue5 were confirmed by PCR for the presence of CLCuGeV, CLCuGeB and OYCrCMA. Thus, from all these analysis, it can be concluded for the first time that there is an unusual degree of complexity of begomoviruses, associated betasatellites and alphasatellites in okra plants in Cameroon. Presence of such a complex in cultivated crops may result in recombination between components and increased host range. The begomovirus/satellite complex may then invade new hosts and pose a serious challenge to other cultivated crops in the neighboring areas.

3.1.3 Co-inoculation of begomoviruses and their associated satellites to assess the compatibility of viruses and satellites from different geographic regions.

As has been described in the previous sections, leaf curl diseases in Asia and Africa are caused by complexes of begomoviruses associated with DNA satellites. A detailed study was carried out to test the possibility of functional interactions between begomoviruses and DNA satellites from different geographical regions, to assess their compatibility. Partial tandem repeats of begomoviruses and/or their associated satellites were constructed to be used in this study **(Paper-I&III)**. *N. benthamiana* plants were agro-inoculated using *Agrobacterium tumefaciens*. To test the compatibility of begomoviruses and satellites from Asia, Africa and Mediterranean Region, different combinations of monopartite begomoviruses and satellites were analysed in infection experiments: Cotton leaf curl Kokhran virus (CLCuKoV), CLCuMuV along with their associated CLCuMB from Pakistan; OYCrV, CLCuGeB, ALCCMBand ALCCMA from Cameroon; TYLCV-IL from Mediterranean Region. Inoculation with either CLCuKoV or CLCuMuV resulted in mild symptoms (Severity index 2), but together with their cognate CLCuMB the symptoms became highly enhanced (Severity index 4) at 14 dpi. Both virus and betasatellite accumulated to high level. After inoculation of plants with CLCuKoV or CLCuMuV with CLCuGeB, the plants exhibited symptoms of leaf curling, leaf crumpling, vein yellowing and stunted growth, but with no vein thickening. The virus accumulated to a higher level as compared to virus alone in both combinations, but in case of CLCuKoV with CLCuGeB the virus

titer was higher than in combination with CLCuMB. In comparison, for inoculation of CLCuKoV or CLCuMuV with non-cognate ALCCMB, the symptoms were reduced and the betasatellite did not accumulate to a high level. It was found that the two Asian begomoviruses did not support ALCCMB very well, because the betasatellite could be detect by PCR but Southern blot hybridization showed that the betasatellite level was low. Both cognate CLCuMB and non-cognate CLCuGeB were supported well by both Asian begomoviruses and they accumulated to high levels as assessed by Southern hybridization. For co-inoculation of CLCuKoV with ALCCMA, severe symptoms were observed and both alphasatellite and virus accumulated to high levels. However, co-inoculation of CLCuMuV with ALCCMA did not result in any significant difference in virus accumulation.

All plants inoculated with OYCrV remained symptomless, however, when co-inoculated with non-cognate CLCuMB the symptom severity was highly enhanced with leaf curling and vein thickening but no leaf enation. OYCrV accumulation was then also increased as compared to OYCrV alone. This is probably because CLCuMB encodes βC1, which acts as a strong silencing suppressor of PTGS (Qazi *et al.*, 2007). Inoculation of OYCrV with CLCuGeB produced delayed symptoms at 21 dpi. Moreover, the accumulation of OYCrV and CLCuGeB was fairly low. Similarly, co-inoculation of OYCrV and ALCCMB also produced delayed symptoms, but the symptom severity was higher than for the combination of OYCrV and CLCuGeB. OYCrV was also co-inoculated with ALCCMB and ALCCMA together. Interestingly, the level of OYCrV was higher when co-inoculated with ALCCMB as compared to when ALCCMA also was present. The ALCCMA titer was low when only inoculated together with OYCrV, but it increased when ALCCMB was present. Inoculation of TYLCV-IL induced typical leaf curl symptoms at 14 dpi and virus accumulated to very high level in systemic leaves. When co-inoculated with CLCuMB symptoms became visible at 10-12 dpi, characterized by typical CLCuMB symptoms. The betasatellite titer was high, but the virus titer was very low as compared to TYLCV-IL alone, suggesting a competition between betasatellite and helper virus accumulation. Inoculation of TYLCV-IL and CLCuGeB induced symptoms at 12 dpi with high severity (Severity index 5) and both helper virus and betasatellite accumulated to higher levels. In combination with ALCCMB the plants started showing symptoms at 16 dpi. Similar to the combination with CLCuMB, ALCCMB accumulated to a high level, and helper virus accumulation was affected. Thus, ALCCMB also competes with the helper begomovirus, a phenomenon occasionally has been observed for begomovirus-satellite complexes. With TYLCV-IL in combination with ALCCMA the plants started showing symptoms at 14 dpi,

but the symptoms were similar to TYLCV-IL alone. The virus accumulation was higher as compared to the combination of TYLCV-IL with CLCuMB or ALCCMB.

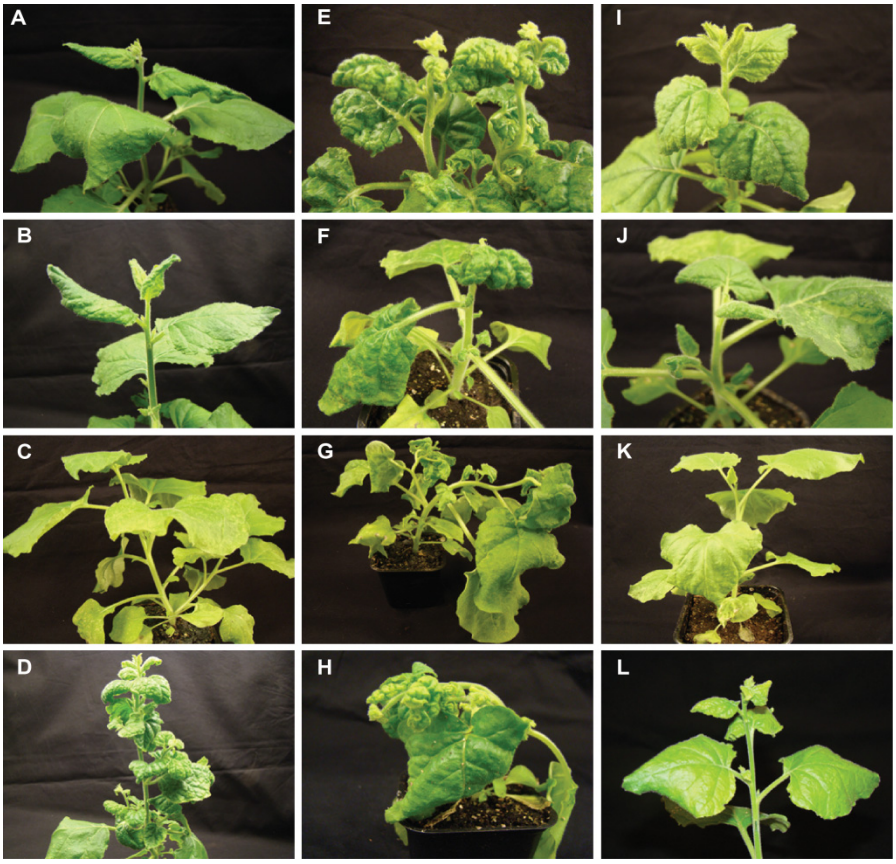

Figure 3. Nicotiana benthamiana plants inoculated with CLCuKoV (A), CLCuMuV (B), OYCrV (C) and TYLCV-IL (D) alone, with CLCuMB (E-H) and with ALCCMA (I-L). When inoculated alone begomoviruses gave mild curling or severe curling (TYLCV-IL). The symptoms get enhanced with CLCuMB and remain same with ALCCMA.

The results demonstrated that CLCuMB, CLCuGeB and ALCCMB are very flexible in their interactions with new non-cognate helper viruses. Thus, these betasatellites may become established in new geographical regions through cross-border trade or any other means.

It was also found that betasatellites compete with each other and/or with helper begomovirus when present within the same plant. Co-inoculation of CLCuMuV with CLCuMB and CLCuGeB induced vein thickening, without

leaf enations on the underside of the leaves. Instead plants were showing leaf crumpling and vein yellowing; characteristic symptoms of CLCuGeB. Both betasatellites showed reduced accumulation within the same plant when inoculated together, while the accumulation of CLCuMuV was enhanced. When CLCuMuV was co-inoculated with CLCuMB and ALCCMB, the accumulation of ALCCMB was very low. ALCCMB was detected by PCR, but could not be detected by Southern blot hybridization.

The non-cognate alphasatellite ALCCMA was well supported and maintained by all begomoviruses from Asia, Africa and the Mediterranean Region. The alphasatellite was successfully detected in all plants inoculated and accumulated to a high level. In combination with OYCrV and ALCCB, ALCCMA accumulated to high level, which further supports that ALCCMB may act as a strong silencing suppressor. Alphasatellites may help modulate virus proliferation (Patil & Fauquet, 2010) and/or may help in silencing suppression (Nawaz-ul-Rehman *et al.*, 2010). In the present study both roles of ALCCMA were observed: for CLCuKoV, virus accumulation was increased in the presence of ALCCMA, but for OYCrV, accumulation was significantly decreased.

Thus it is quite possible that an exchange of DNA satellites across the continents may further broaden the host range of begomoviruses prevailing already in the area and thereby leading to emergence of new viral complexes.

3.1.4 Effects of mutation of selected genes of *Cotton leaf curl Kokhran virus* on infectivity, symptoms and maintenance of Cotton leaf curl Multan betasatellite

CLCuKoV is one of the cotton infecting monopartite begomoviruses (family *Geminiviridae*) in Asia. It has six ORFs and it is associated with single betasatellite, CLCuMB (Fig. 1). The virus alone causes mild leaf curling, reduced leaf size and stunted growth of the plants, but co-inoculation with betasatellite results in severe leaf curling and leaf enations on the abaxial side of the leaves (Fig. 3). Several studies have examined the effects of mutagenesis of monopartite begomovirus genes (Rigden *et al.*, 1993), but this is the first such study on a monopartite, malvaceae-adapted and betasatellite associated begomovirus **(Paper-IV)**. Mutations were introduced into the *CP*, *V2*, *C2* and *C4* genes of CLCuKoV, and infectious clones were constructed containing these mutations. CLCuKoV with a mutated *V2* gene neither induced symptoms in *N. benthamiana* nor efficiently maintained CLCuMB. Southern blot analysis showed greatly reduced viral levels in systemic leaves, indicating a reduced efficiency of viral movement for the V2 mutant. The *V2* mutation could not be complemented by overexpression of *V2* under the control of the CaMV 35S

promoter in the inoculated leaves. V2 also acts as a suppressor of PTGS and may then possibly suppress host PTGS-based resistance to virus movement. Begomoviruses native to the NW lack the *V2* gene, suggesting that this may be the reason why this region lacks monopartite begomoviruses. Inoculation of plants with the CP mutant did not result in symptoms and thus abolished infectivity of CLCuKoV. Moreover, betasatellite could not restore infectivity, but with overexpression of *CP* under 35S promoter the infection could be complemented in more plants, in comparison to plants inoculated with only mutant and *CP* expressed under 35S promoter. It suggested that CP is required by the virus to get access to the phloem and/or to establish infection in the young tissues. Moreover, CP is also a requirement for maintenance of betasatellite. In the plants inoculated with CLCuKoV mutated for *C2* gene did not show any symptoms and the virus could be detected only by PCR in 4 out of 10 plants. Co-inoculation with CLCuMB restored infectivity in a high proportion of the plants (9 out of 10 inoculated). The betasatellite was not maintained in all infected plants, but it was obvious that betasatellite was able to complement the *C2* mutation. Transient expression of C2 together with the inoculation with the C2 mutant of CLCuKoV and/or CLCuMuB did not induce any symptoms but increased virus DNA level in the distal leaves. Moreover, plants with transient expression of 35S-C2 alone showed chlorosis at site of inoculation. It is likely that the cell-death induced by C2 overexpression prevented virus and/or betasatellite moving out of the inoculated patch of the leaf tissue. The C4 protein of monopartite begomoviruses and curtoviruses takes part in symptom modulation of the virus. All *N. benthamiana* plants inoculated with th C4 mutant of CLCuKoV showed 1-2 days delayed symptoms as compared to wild type CLCuKoV and the plants did not develop any vein thickening. These results suggest that the C4 protein is not necessary for viral replication, but may take part in virus spread, modulation of symptoms and induction of cell proliferation. The virus titer of the C4 mutant was almost similar to that of wild type CLCuKoV. Co-inoculation with C4 mutant of CLCuKoV and CLCuMB induced symptoms equivalent to wild type CLCuKoV alone. The virus as well as betasatellite could accumulate to high levels similar to wild type CLCuKoV. This may suggest that both the C4 protein of CLCuKoV and βC1 of CLCuMuV induce hyperplasia. Transiently expressed C4 together with inoculation of the C4 mutant of CLCuKoV and CLCuMB induced symptoms at 11-12 dpi. Vein thickening symptoms then also developed and the CLCuKoV level was similar to wild type. Thus, complementation with a *C4* gene (35S-C4) had no effect on neither infectivity nor viral/betasatellite levels in the inoculated plants. It is clear that C4 is not absolutely required for maintenance of the betasatellite. The results of this

study are quite consistent with previous studies, suggesting that the CP and V2 protein play a role in virus movement and that C2 and C4 are pathogenicity determinant. It is also obvious for the first time that apart from Rep, the V2 and C2 proteins play an important role in maintaining the betasatellite. It will be interesting to further investigate the effects of mutations in these genes on the second type of satellites, alphasatellites.

4 Conclusions

The main conclusions from the results presented in this thesis are.

1. Okra in Cameroon is a host to at least three begomoviruses: CLCuGeV also found in Burkina Faso, Egypt, Mali and Sudan; OYCrV also found in Mali; and OLCuCMV a new recombinant species from Cameroon. These viruses were associated with DNA satellites: CLCuGeB also found in above mentioned African countries; CLCuGeA also found in Burkina Faso and Mali and OYCrCMA also found in Burkina Faso. A mixed infection of OYCrV and OLCuCMV was also found in two okra plants. The results suggest that okra in Africa is a host to many begomovirus complexes, possibly yet un-identified variants and/or species.
2. *A. conyzoides* (Goat weed) in Cameroon is a host of a new begomovirus complex comprising of a new begomovirus species ALCCMV, a new betasatellite species, ALCCMB and a new alphasatellite species, ALCCMA. In addition, two fragments containing begomovirus-alphasatellite sequences were also sequenced referred to as defective interfering molecules. Thus begomovirus-satellite complexes infecting weeds in Cameroon may be more complex with uncharacterized species and/strains.
3. The diversity of begomoviruses and associated DNA-satellites in Africa is much higher than previously thought. It may be similar to the begomovirus diversity prevailing in Asia.
4. Possibly, there are still many un-characterized species in Africa, which needs further investigation to be explored.
5. The begomoviruses from different geographical regions can potentially interact with the non-cognate betasatellites and alphasatellites with varying efficiencies. Thus it is quite possible that exchange of satellites may occur among begomoviruses across the continents, which may further broaden the host range of begomoviruses already present in the area.

6. Begomoviruses and satellites from Africa, Asia and Mediterranean Region have been separated for a long time and thus are more distant from each other, but they can still support and interact with each other.
7. Begomovirus genes *V2*, *C2* and *CP* are pathogenicity determinants and are important for the maintenance of betasatellites by monopartite begomoviruses. *C4* is responsible for symptom induction, but it is not required for maintenance of betasatellites.
8. The interactions between helper viruses and betasatellites are much more complex than previously suggested.

5 Future perspectives

- From the survey conducted on ageratum and okra in Cameroon, it is quite evident that the complexity of begomoviruses and DNA-satellites in this region is high, and our knowledge needs to be broadened further with more extensive surveys including a wider range of geographical areas.
- There is a need to investigate alternative hosts, other than crops, for the begomoviruses and satellites in Africa.
- There is an urgent need to introduce or breed crops with broad-spectrum resistance to cope with possible emergence of new begomovirus complexes all over the world particularly in Asia and Africa.
- There is a need to investigate the effects of mutations of virus-encoded genes on the second class of begomovirus-associated satellites, the alphasatellites.

References

Ahmed, M., Ren, S.-X., Mandour, N., Maruthi, M., Naveed, M. & Qiu, B.-L. (2010). Phylogenetic analysis of *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations from cotton plants in Pakistan, China, and Egypt. *Journal of Pest Science* 83(2), 135-141.

Alberter, B., Rezaian, M.A. & Jeske, H. (2005). Replicative intermediates of *Tomato leaf curl virus* and its satellite DNAs. *Virology* 331, 441-448.

Amin, I., Hussain, K., Akbergenov, R., Yadav, J.S., Qazi, J., Mansoor, S., Hohn, T., Fauquet, C.M. & Briddon, R.W. (2011a). Suppressors of RNA silencing encoded by the components of the cotton leaf curl begomovirus-betasatellite complex. *Mol Plant Microbe Interact* 24(8), 973-83.

Amin, I., Patil, B., Briddon, R., Mansoor, S. & Fauquet, C. (2011b). A common set of developmental miRNAs are upregulated in Nicotiana benthamiana by diverse begomoviruses. *Virology Journal* 8(1), 143.

Amin, I., Patil, B.L., Briddon, R.W., Mansoor, S. & Fauquet, C.M. (2011c). Comparison of phenotypes produced in response to transient expression of genes encoded by four distinct begomoviruses in Nicotiana benthamiana and their correlation with the levels of developmental miRNAs. *Virology Journal* 8, 238.

Anbinder, I., Reuveni, M., Azari, R., Paran, I., Nahon, S., Shlomo, H., Chen, L., Lapidot, M. & Levin, I. (2009). Molecular dissection of Tomato leaf curl virus resistance in tomato line TY172 derived from Solanum peruvianum. *Theoretical and Applied Genetics* 119(3), 519-30.

Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R. & Daszak, P. (2004). Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology and Evolution* 19(10), 535-544.

Arguello-Astorga, G., Ascencio-Ibanez, J.T., Dallas, M.B., Orozco, B.M. & Hanley-Bowdoin, L. (2007). High frequency reversion of geminivirus replication protein mutants during infection. *Journal of Virology* 81(20), 11005-11015.

Azhar, M.T., Amin, I., Anjum, Z.I., Arshad, M., Briddon, R.W. & Mansoor, S. (2010). Both malvaceous and non-malvaceous betasatellites are associated

with two wild cotton species grown under field conditions in Pakistan. *Virus Genes* 41(3), 417-424.

Azzam, O., Frazer, J., de la Rosa, D., Beaver, J.S., Ahlquist, P. & Maxwell, D.P. (1994). Whitefly transmission and efficient ssDNA accumulation of bean golden mosaic geminivirus requires functional coat protein. *Virology* 204, 289-296.

Bagewadi, B., Chen, S., Lal, S.K., Choudhury, N.R. & Mukherjee, S.K. (2004). PCNA interacts with Indian mung bean Yellow mosaic virus Rep and downregulates Rep activity. *Journal of Virology* 78(21), 11890-11903.

Baumann, P., Munson, M.A., Lai, C.Y., Clark, M.A., Baumann, L., Moran, N.A. & Campbell, B.C. (1993). Origin and properties of bacterial endosymbionts of aphids, whiteflies, and mealybugs. *ASM News* 59, 21-24.

Bedford, I.D., Briddon, R.W., Brown, J.K., Rosell, R.C. & Markham, P.G. (1994). Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Annals of Applied Biology* 125(2), 311-325.

Bisaro, D. (1996). Geminivirus DNA replication. In: *DNA replication in eukaryotic cells*. pp. 833-854 Cold Spring Harbor Laboratory \press.

Bisaro, D.M. (2006). Silencing suppression by geminivirus proteins. *Virology* 344, 158-168.

Bivalkar-Mehla, S., Vakharia, J., Mehla, R., Abreha, M., Kanwar, J.R., Tikoo, A. & Chauhan, A. (2011). Viral RNA silencing suppressors (RSS): Novel strategy of viruses to ablate the host RNA interference (RNAi) defense system. *Virus Research* 155(1), 1-9.

Blawid, R., Van, D.T. & Maiss, E. (2008). Transreplication of a Tomato yellow leaf curl Thailand virus DNA-B and replication of a DNAβ component by Tomato leaf curl Vietnam virus and Tomato yellow leaf curl Vietnam virus. *Virus Research* 136(1-2), 107-117.

Bosco, D., Mason, G. & Accotto, G.P. (2004). TYLCSV DNA, but not infectivity, can be transovarially inherited by the progeny of the whitefly vector *Bemisia tabaci* (Gennadius). *Virology* 323, 276-283.

Bradeen, J.M., Timmermans, M.C. & Messing, J. (1997). Dynamic genome organization and gene evolution by positive selection in geminivirus (Geminiviridae). *Molecular Biology and Evolution* 14(11), 1114-24.

Briddon, R.W., Bedford, I.D., Tsai, J.H. & Markham, P.G. (1996). Analysis of the nucleotide sequence of the treehopper-transmitted geminivirus, tomato pseudo-curly top virus, suggests a recombinant origin. *Virology* 219(2), 387-394.

Briddon, R.W., Brown, J.K., Moriones, E., Stanley, J., Zerbini, M., Zhou, X. & Fauquet, C.M. (2008). Recommendations for the classification and nomenclature of the DNA-β satellites of begomoviruses. *Archives of Virology* 153(4), 763-781.

Briddon, R.W., Bull, S.E., Amin, I., Idris, A.M., Mansoor, S., Bedford, I.D., Dhawan, P., Rishi, N., Siwatch, S.S., Abdel-Salam, A.M., Brown, J.K., Zafar, Y. & Markham, P.G. (2003). Diversity of DNA β: a satellite molecule associated with some monopartite begomoviruses. *Virology* 312(1), 106-121.

Briddon, R.W., Bull, S.E., Amin, I., Mansoor, S., Bedford, I.D., Rishi, N., Siwatch, S.S., Zafar, M.Y., Abdel-Salam, A.M. & Markham, P.G. (2004). Diversity of DNA 1; a satellite-like molecule associated with monopartite begomovirus-DNA β complexes. *Virology* 324(2), 462-474.

Briddon, R.W., Mansoor, S., Bedford, I.D., Pinner, M.S. & Markham, P.G. (2000). Clones of cotton leaf curl geminivirus induce symptoms atypical of cotton leaf curl disease. *Virus Genes* 20(1), 17-24.

Briddon, R.W., Mansoor, S., Bedford, I.D., Pinner, M.S., Saunders, K., Stanley, J., Zafar, Y., Malik, K.A. & Markham, P.G. (2001). Identification of DNA components required for induction of cotton leaf curl disease. *Virology* 285(2), 234-243.

Briddon, R.W. & Markham, P.G. (2001). Cotton leaf curl virus disease. *Virus Research* 71(1-2), 151-159.

Briddon, R.W. & Stanley, J. (2006). Sub-viral agents associated with plant single-stranded DNA viruses. *Virology* 344(1), 198-210.

Brown, J.K. *The Bemisia tabaci complex: genetic and phenotypic variability drives begomovirus spread and virus diversification. http://www.apsnet.org/online/feature/btabaci/*. [online] Available from: http://www.apsnet.org/online/feature/btabaci/.

Brown, J.K., Fauquet, C.M., Briddon, R.W., Zerbini, M., Moriones, E. & Navas-Castillo, J. (2012). Family *Geminiviridae*. In: A. M. Q. King, M. J. Adams, E. B. Carstens & E. J. Lefkowitz (Ed), Virus Taxonomy: Classification and Nomenclature of Viruses - Ninth Report of the International Committee on Taxonomy of Viruses. In: Elsevier Academic Press, USA, pp. 351-373.

Brown, J.K. & Idris, A.M. (2005). Genetic differentiation of whitefly Bemisia tabaci mitochondrial cytochrome oxidase I, and phylogeographic concordance with the coat protein of the plant virus genus Begomovirus. *Annals of the Entomological Society of America* 98(6), 827-837.

Bull, S.E., Briddon, R.W., Sserubombwe, W.S., Ngugi, K., Markham, P.G. & Stanley, J. (2006). Genetic diversity and phylogeography of cassava mosaic viruses in Kenya. *Journal of General Virology* 87(10), 3053-3065.

Bull, S.E., Tsai, W.-S., Briddon, R.W., Markham, P.G., Stanley, J. & Green, S.K. (2004). Diversity of begomovirus DNA β satellites of non-malvaceous plants in east and south east Asia. *Archives of Virology* 149(6), 1193 - 1200.

Cerutti, H. & Casas-Mollano, J.A. (2006). On the origin and functions of RNA-mediated silencing: From protists to man. *Current Genetics* 50(2), 81-99.

Cheng, X., Wang, X., Wu, J., Briddon, R.W. & Zhou, X. (2011). βC1 encoded by tomato yellow leaf curl China betasatellite forms multimeric complexes in vitro and in vivo. *Virology* 409(2), 156-62.

Choudhury, N.R., Malik, P.S., Singh, D.K., Islam, M.N., Kaliappan, K. & Mukherjee, S.K. (2006). The oligomeric Rep protein of Mungbean yellow mosaic India virus (MYMIV) is a likely replicative helicase. *Nucleic Acids Research* 34(21), 6362-6377.

Colvin, J., Omongo, C.A., Maruthi, M.N., Otim-Nape, G.W. & Thresh, J.M. (2004). Dual begomovirus infections and high *Bemisia tabaci*

populations: two factors driving the spread of a cassava mosaic disease pandemic. *Plant Pathology* 53, 577-584.

Cui, X., Li, G., Wang, D., Hu, D. & Zhou, X. (2005). A begomovirus DNAβ-encoded protein binds DNA, functions as a suppressor of RNA silencing, and targets the cell nucleus. *Journal of Virology* 79, 10764-10775.

Czosnek, H., Ghanim, M. & Ghanim, M. (2002). The circulative pathway of begomoviruses in the whitefly vector *Bemisia tabaci* — insights from studies with *Tomato yellow leaf curl virus*. *Annals of Applied Biology* 140, 215-231.

De Barro, P.J., Liu, S.S., Boykin, L.M. & Dinsdale, A.B. (2011). Bemisia tabaci: a statement of species status. *Annual Review of Entomology* 56, 1-19.

De Barro, P.J., Trueman, J.W. & Frohlich, D.R. (2005). *Bemisia argentifolii* is a race of *B. tabaci* (Hemiptera: Aleyrodidae): the molecular genetic differentiation of *B. tabaci* populations around the world. *Bulletin of Entomological Research* 95, 193-203.

Ding, S.W. & Voinnet, O. (2007). Antiviral immunity directed by small RNAs. *Cell* 130(3), 413-426.

Domingo, E. & Holland, E.E. (1997). RNA virus mutations and fitness for survival. *Annual Review of Microbiology* 51, 151-178.

Dry, I., Krake, L.R., Rigden, J.E. & Rezaian, M.A. (1997). A novel subviral agent associated with a geminivirus: the first report of a DNA satellite. *Proceedings of the National Academy of Sciences USA* 94, 7088-7093.

Duffy, S. & Holmes, E.C. (2008). Phylogenetic evidence for rapid rates of molecular evolution in the single-stranded DNA begomovirus Tomato yellow leaf curl virus (TYLCV). *Journal of Virology* 82(2), 957-965.

Duffy, S. & Holmes, E.C. (2009). Validation of high rates of nucleotide substitution in geminiviruses: phylogenetic evidence from East African cassava mosaic viruses. *Journal of General Virology* 90(Pt 6), 1539-47.

Dunoyer, P., Lecellier, C.-H., Parizotto, E.A., Himber, C. & Voinnet, O. (2004). Probing the microRNA and small interfering RNA pathways with virus-encoded suppressors of RNA silencing. *Plant Cell* 16(5), 1235-1250.

Eini, O., Dogra, S., Selth, L.A., Dry, I.B., Randles, J.W. & Rezaian, M.A. (2009). Interaction with a host ubiquitin-conjugating enzyme is required for the pathogenicity of a geminiviral DNA beta satellite. *Mol Plant Microbe Interact* 22(6), 737-46.

Fargette, D., Konate, G., Fauquet, C., Muller, E., Peterschmitt, M. & Thresh, J.M. (2006). Molecular ecology and emergence of tropical plant viruses. *Annual Review of Phytopathology* 44, 235-260.

Fauquet, C. & Stanley, J. (2003). Geminivirus classification and nomenclature: progress and problems. *Annals of Applied Biology* 142, 165-189.

Fauquet, C.M., Bisaro, D.M., Briddon, R.W., Brown, J.K., Harrison, B.D., Rybicki, E.P., Stenger, D.C. & Stanley, J. (2003). Revision of taxonomic criteria for species demarcation in the family *Geminiviridae*, and an updated list of begomovirus species. *Archives of Virology* 148(2), 405-421.

Fauquet, C.M., Briddon, R.W., Brown, J.K., Moriones, E., Stanley, J., Zerbini, M. & Zhou, X. (2008). Geminivirus strain demarcation and nomenclature. *Archives of Virology* 153(4), 783-821.

Fauquet, C.M., Sawyer, S., Idris, A.M. & Brown, J.K. (2005). Sequence analysis and classification of apparent recombinant begomoviruses infecting tomato in the Nile and Mediterranean Basins. *Phytopathology* 95, 549-555.

Fauquet, C.M. & Stanley, J. (2005). Revising the way we conceive and name viruses below the species level: A review of geminivirus taxonomy calls for new standardized isolate descriptors. *Archives of Virology* 150, 2151-2179.

Fondong, V.N. & Chen, K. (2011). Genetic variability of East African cassava mosaic Cameroon virus under field and controlled environment conditions. *Virology* 413(2), 275-282.

Fontes, E.P.B., Santos, A.A., Luz, D.F., Waclawovsky, A.J. & Chory, J. (2004). The geminivirus nuclear shuttle protein is a virulence factor that suppresses transmembrane receptor kinase activity. *Genes and Development* 18(20), 2545-2556.

Gafni, Y. & Epel, B.L. (2002). The role of host and viral proteins in intra- and inter-cellular trafficking of geminiviruses. *Physiological and Molecular Plant Pathology* 60, 231-241.

García-Andrés, S., Accotto, G.P., Navas-Castillo, J. & Moriones, E. (2007a). Founder effect, plant host, and recombination shape the emergent population of begomoviruses that cause the tomato yellow leaf curl disease in the Mediterranean basin. *Virology* 359(2), 302-312.

García-Andrés, S., Tomás, D.M., Sánchez-Campos, S., Navas-Castillo, J. & Moriones, E. (2007b). Frequent occurrence of recombinants in mixed infections of tomato yellow leaf curl disease-associated begomoviruses. *Virology* 365(1), 210-219.

Ge, L., Zhang, J., Zhou, X. & Li, H. (2007). Genetic structure and population variability of *Tomato yellow leaf curl China virus*. *Journal of Virology* 81(11), 5902-5907.

Gladfelter, H.J., Eagle, P.A., Fontes, E.P.B., Batts, L. & Hanley-Bowdoin, L. (1997). Two domains of the AL1 protein mediate geminivirus origin recognition. *Virology* 239, 186-197.

Glick, E., Zrachya, A., Levy, Y., Mett, A., Gidoni, D., Belausov, E., Citovsky, V. & Gafni, Y. (2007). Interaction with host SGS3 is required for suppression of RNA silencing by tomato yellow leaf curl virus V2 protein. *Proceedings of the National Academy of Sciences USA* 105(1), 157-161.

Goodman, R.M. (1977). Infectious DNA from a whitefly-transmitted virus of *Phaseolus vulgaris*. *Virology* 266, 54-55.

Gopal, P., Kumar, P., Sinilal, B., Jose, J., Kasin Yadunandam, A. & Usha, R. (2007). Differential roles of C4 and βC1 in mediating suppression of post-transcriptional gene silencing: Evidence for transactivation by the C2 of Bhendi yellow vein mosaic virus, a monopartite begomovirus. *Virus Research* 123(1), 9-18.

Gutierrez, C. (2000). Geminiviruses and the plant cell cycle. *Plant Molecular Biology* 43, 763–772.

Hanley-Bowdoin, L., Settlage, S.B., Orozco, B.M., Nagar, S. & Robertson, D. (1999). Geminviruses: models for plant DNA replication, transcription, and cell cycle regulation. *Critical Reviews in Plant Sciences* 18, 71-106.

Hanley-Bowdoin, L., Settlage, S.B. & Robertson, D. (2004). Reprogramming plant gene expression: a prerequisite to geminivirus DNA replication. *Molecular Plant Pathology* 5, 149-156.

Harlan, J.R. (1971). Agricultural origins: centres and non centres. *Science* 174, 468–474.

Harrison, B.D., Barker, H., Bock, K.R., Guthrie, E.J., Meredith, G. & Atkinson, M. (1977). Plant viruses with circular single-stranded DNA. *Nature* 270, 760-762.

Hong, Y.G., Robinson, D.J. & Harrison, B.D. (1993). Nucleotide sequence evidence for the occurrence of three distinct whitefly-transmitted geminiviruses in cassava. *Journal of General Virology* 74, 2437-2443.

Huang, J.F. & Zhou, X.P. (2006). Molecular characterization of two distinct begomoviruses from *Ageratum conyzoides* and *Malvastrum coromandelianum* in China. *Journal of Phytopathology* 154(11-12), 648–653.

Hull, R. (2002). *Matthews' Plant Virology*. 4. ed. San Diego: Academic Press.

Hussain, M., Mansoor, S., Iram, S., Fatima, A.N. & Zafar, Y. (2005). The nuclear shuttle protein of *Tomato leaf curl New Delhi virus* is a pathogenicity determinant. *Journal of Virology* 79, 4434–4439.

Hussain, M., Mansoor, S., Iram, S., Zafar, Y. & Briddon, R.W. (2007). The hypersensitive response to tomato leaf curl New Delhi virus nuclear shuttle protein is inhibited by transcriptional activator protein. *Mol Plant Microbe Interact* 20(12), 1581-8.

Idris, A.M., Briddon, R.W., Bull, S.E. & Brown, J.K. (2005). Cotton leaf curl Gezira virus-satellite DNAs represent a divergent, geographically isolated Nile Basin lineage: predictive identification of a satDNA REP-binding motif. *Virus Research* 109(1), 19-32.

Idris, A.M. & Brown, J.K. (2002). Molecular analysis of cotton leaf curl virus-Sudan reveals an evolutionary history of recombination. *Virus Genes* 24, 249-256.

Idris, A.M. & Brown, J.K. (2004). *Cotton leaf crumple virus* is a distinct Western Hemisphere begomovirus species with complex evolutionary relationships indicative of recombination and reassortment. *Phytopathology* 94, 1068-1074.

Idris, A.M. & Brown, J.K. (2005). Evidence for interspecific-recombination for three monopartite begomoviral genomes associated with the tomato leaf curl disease from central Sudan. *Archives of Virology* 150(5), 1003-1012.

Idris, A.M., Shahid, M.S., Briddon, R.W., Khan, A.J., Zhu, J.-K. & Brown, J.K. (2011). An unusual alphasatellite associated with monopartite begomoviruses attenuates symptoms and reduces betasatellite accumulation. *Journal of General Virology* 92, 706-717.

Ito, T., Kimbara, J., Sharma, P. & Ikegami, M. (2009). Interaction of tomato yellow leaf curl virus with diverse betasatellites enhances symptom severity. *Archives of Virology* 154(8), 1233-1239.

Jeffrey, J.L., Pooma, W. & Petty, I.T.D. (1996). Genetic requirements for local and systemic movement of tomato golden mosaic virus in infected plants. *Virology* 223, 208-218.

Jenkins, G.M., Rambaut, A., Pybus, O.G. & Holmes, E.C. (2002). Rates of molecular evolution in RNA viruses: a quantitative phylogenetic analysis. *Journal of Molecular Evolution* 54(2), 156-65.

Jeske, H. (2007). Replication of geminiviruses and the use of rolling circle amplification for their diagnosis. In: *Czosnek, H. (Ed.), Tomato Yellow Leaf Curl Virus Disease pp. 141-156* Springer, Dordrecht.

Jiu, M., Zhou, X., Tong, L., Xu, J., Yang, X., Wan, F. & Liu, S. (2007). Vector-virus mutualism accelerates population increase of an invasive whitefly. *PLoS ONE* 2 e182.

Jiu, M., Zhou, X.P. & Liu, S.S. (2006). Acquisition and transmission of two begomoviruses by the B and a non-B biotype of *Bemisia tabaci* from Zhejiang, China. *Journal of Phytopathology* 154, 587-591.

Jones, R.A.C. (2009). Plant virus emergence and evolution: Origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. *Virus Research* 141(2), 113-130.

Kaliappan, K., Choudhury, N.R., Suyal, G. & Mukherjee, S.K. (2012). A novel role for RAD54: this host protein modulates geminiviral DNA replication. *FASEB J* 26(3), 1142-60.

Kirthi, N., Priyadarshini, C.G.P., Sharma, P., Maiya, S.P., Hemalatha, V., Sivaraman, P., Dhawan, P., Rishi, N. & Savithri, H.S. (2004). Genetic variability of begomoviruses associated with cotton leaf curl disease originating from India. *Archives of Virology* 149, 2047-2057.

Kon, T., Hidayat, S.H., Hase, S., Takahashi, H. & Ikegami, M. (2006). The natural occurrence of two distinct begomoviruses associated with DNA β and a recombinant DNA in a tomato plant from Indonesia. *Phytopathology* 96, 517-525.

Kon, T., Kuwabara, K., Hidayat, S.H. & Ikegami, M. (2007). A begomovirus associated with ageratum yellow vein disease in Indonesia: evidence for natural recombination between tomato leaf curl Java virus and Ageratum yellow vein virus-[Java]. *Archives of Virology* 152(6), 1147-1157.

Kon, T., Rojas, M.R., Abdourhamane, I.K. & Gilbertson, R.L. (2009). Roles and interactions of begomoviruses and satellite DNAs associated with okra leaf curl disease in Mali, West Africa. *Journal of General Virology* 90(Pt 4), 1001-13.

Koonin, E.V. & Ilyina, T.V. (1992). Geminivirus replication proteins are related to prokaryotic plasmid rolling circle DNA replication initiator proteins. *Journal of General Virology* 73, 2763-2766.

Kumar, P.P., Usha, R., Zrachya, A., Levy, Y., Spanov, H. & Gafni, Y. (2006). Protein-protein interactions and nuclear trafficking of coat protein and βC1 protein associated with Bhendi yellow vein mosaic disease. *Virus Research* 122(1-2), 127-136.

Lefeuvre, P., Lett, J.-M., Varsani, A. & Martin, D.P. (2009). Widely conserved recombination patterns amongst single stranded DNA viruses. *Journal of Virology* 83, 2697-2707.

Lefeuvre, P., Martin, D.P., Hoareau, M., Naze, F., Delatte, H., Thierry, M., Varsani, A., Becker, N., Reynaud, B. & Lett, J.M. (2007). Begomovirus 'melting pot' in the south-west Indian Ocean islands: molecular diversity and evolution through recombination. *Journal of General Virology* 88(12), 3458-3468.

Legg, J.P. & Fauquet, C.M. (2004). Cassava mosaic geminiviruses in Africa. *Plant Molecular Biology* 56, 585-599.

Legg, J.P., Jeremiah, S.C., Obiero, H.M., Maruthi, M.N., Ndyetabula, I., Okao-Okuja, G., Bouwmeester, H., Bigirimana, S., Tata-Hangy, W., Gashaka, G., Mkamilo, G., Alicai, T. & Lava Kumar, P. (2011). Comparing the regional epidemiology of the cassava mosaic and cassava brown streak virus pandemics in Africa. *Virus Research* 159(2), 161-170.

Leke, W.N., Brown, J.K., Ligthart, M.E., Sattar, N., Njualem, D.K. & Kvarnheden, A. (2012). *Ageratum conyzoides*: A host to a unique begomovirus disease complex in Cameroon. *Virus Research* 163(1), 229-37.

Leke, W.N., Kvarnheden, A., Ngane, E.B., Titanji, V.P. & Brown, J.K. (2011). Molecular characterization of a new begomovirus and divergent alphasatellite from tomato in Cameroon. *Archives of Virology* 156(5), 925-8.

Lin, B., Behjatnia, A.A., Dry, I.B., Randles, J.W. & Rezaian, M.A. (2003). High-affinity Rep-binding is not required for the replication of a geminivirus DNA and its satellite. *Virology* 305, 353 –363.

Lisha, V.S., Antony, B., Palaniswami, M.S. & Henneberry, T.J. (2003). *Bemisia tabaci* (*Homoptera: Aleyrodidae*) biotypes in India. *Journal of Economic Entomology* 96(2), 322-7.

Lodish, H.F., Zhou, B., Liu, G. & Chen, C.Z. (2008). Micromanagement of the immune system by microRNAs. *Nature Reviews Immunology* 8(2), 120-130.

Lucas, W.J. (2006). Plant viral movement proteins: Agents for cell-to-cell trafficking of viral genomes. *Virology* 344, 169 - 184.

Luqman, A. (2010). *Characterization and infectivity analysis of begomovirus components found in cotton-based agro-ecosystem.* Diss. Islamabad:Quaid-i-Azam University.

MacLean, D., Elina, N., Havecker, E.R., Heimstaedt, S.B., Studholme, D.J. & Baulcombe, D.C. (2010). Evidence for large complex networks of plant short silencing RNAs. *PLoS ONE* 5(3), e9901.

Malik, P.S., Kumar, V., Bagewadi, B. & Mukherjee, S.K. (2005). Interaction between coat protein and replication initiation protein of *Mung bean yellow mosaic India virus* might lead to control of viral DNA replication. *Virology* 337, 273-283

Mansoor, S., Briddon, R.W., Bull, S.E., Bedford, I.D., Bashir, A., Hussain, M., Saeed, M., Zafar, M.Y., Malik, K.A., Fauquet, C. & Markham, P.G. (2003a). Cotton leaf curl disease is associated with multiple monopartite

begomoviruses supported by single DNA β. *Archives of Virology* 148(10), 1969-1986.

Mansoor, S., Briddon, R.W., Zafar, Y. & Stanley, J. (2003b). Geminivirus disease complexes: an emerging threat. *Trends in Plant Science* 8(3), 128-134.

Mansoor, S., Khan, S.H., Bashir, A., Saeed, M., Zafar, Y., Malik, K.A., Briddon, R.W., Stanley, J. & Markham, P.G. (1999). Identification of a novel circular single-stranded DNA associated with cotton leaf curl disease in Pakistan. *Virology* 259(1), 190-199.

Maruthi, M.N., Colvin, J., Seal, S., Gibson, G. & Cooper, J. (2002). Co-adaptation between cassava mosaic geminiviruses and their local vector populations. *Virus Research* 86, 71-85.

Matranga, C. & Zamore, P.D. (2007). Small silencing RNAs. *Current Biology* 17, R789-R793.

Mendez-Lozano, J., Torres-Pacheco, I., Fauquet, C.M. & Rivera-Bustamante, R. (2003). Interactions between geminiviruses in a naturally occurring mixture: *Pepper huasteco virus* and *Pepper golden mosaic virus*. *Phytopathology* 93(3), 270-277.

Moffat, A.S. (1999). Geminiviruses emerge as serious crop threat. *Science* 286, 1835.

Moissiard, G. & Voinnet, O. (2004). Viral suppression of RNA silencing in plants. *Molecular Plant Pathology* 5, 71-82.

Monci, F., Sanchez-Campos, S., Navas-Castillo, J. & Moriones, E. (2002). A natural recombinant between the geminiviruses *Tomato yellow leaf curl Sardinia virus* and *Tomato yellow leaf curl virus* exhibits a novel pathogenic phenotype and is becoming prevalent in Spanish populations. *Virology* 303, 317-326.

Morales, F.J. & Anderson, P.K. (2001). The emergence and dissemination of whitefly-transmitted geminiviruses in Latin America. *Archives of Virology* 146, 415-441.

Morin, S., Ghanim, M., Sobol, I. & Czosnek, H. (2000). The GroEL protein of the whitefly *Bemisia tabaci* interacts with the coat protein of trasmissible and nontransmissible begomoviruses in the yeast two-hybrid system. *Virology* 276, 404-416.

Morin, S., Ghanim, M., Zeidan, M., Czosnek, H., Verbeek, M. & van den Heuvel, J.F.J.M. (1999). A GroEL homologue from endosymbiotic bacteria of the whitefly *Bemisia tabaci* is implicated in the circulative transmission of *Tomato yellow leaf curl virus*. *Virology* 256, 75-84.

Mubin, M., Akhtar, S., Amin, I., Briddon, R.W. & Mansoor, S. (2011). Xanthium strumarium: a weed host of components of begomovirus-betasatellite complexes affecting crops. *Virus Genes*, 1-8.

Mubin, M., Amin, I., Amrao, L., Briddon, R.W. & Mansoor, S. (2010a). The hypersensitive response induced by the V2 protein of a monopartite begomovirus is countered by the C2 protein. *Molecular Plant Pathology* 11(2), 245-254

Mubin, M., Briddon, R.W. & Mansoor, S. (2009). Complete nucleotide sequence of chili leaf curl virus and its associated satellites naturally infecting potato in Pakistan. *Archives of Virology* 154(2), 365-368.

Mubin, M., Shahid, M.S., Tahir, M.N., Briddon, R.W. & Mansoor, S. (2010b). Characterization of begomovirus components from a weed suggests that begomoviruses may associate with multiple distinct DNA satellites. *Virus Genes* 40(3), 452-457.

Naqvi, A.R. (2010). MicroRNA profiling of tomato leaf curl new delhi virus (tolcndv) infected tomato leaves indicates that deregulation of mir159/319 and mir172 might be linked with leaf curl disease *Virology Journal* 7: 281.

Nash, T.E., Dallas, M.B., Reyes, M.I., Buhrman, G.K., Ascencio-Ibanez, J.T. & Hanley-Bowdoin, L. (2011). Functional Analysis of a Novel Motif Conserved across Geminivirus Rep Proteins. *Journal of Virology* 85(3), 1182-1192.

Navas-Castillo, J., Fiallo-Olive, E. & Sanchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. *Annu Rev Phytopathol* 49, 219-48.

Nawaz-ul-Rehman, M.S. & Fauquet, C.M. (2009). Evolution of geminiviruses and their satellites. *FEBS Letters* 583(12), 1825-1832

Nawaz-ul-Rehman, M.S., Nahid, N., Mansoor, S., Briddon, R.W. & Fauquet, C.M. (2010). Post-transcriptional gene silencing suppressor activity of the alpha-Rep of non-pathogenic alphasatellites associated with begomoviruses. *Virology* 405(2), 300-308.

Norrby, E. (2008). Nobel Prizes and the emerging virus concept. *Archives of Virology* 153(6), 1109-1123.

Obbard, D.J., Gordon, K.H.J., Buck, A.H. & Jiggins, F.M. (2009). The evolution of RNAi as a defence against viruses and transposable elements. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364(1513), 99-115.

Osei, M.K., Akromah, R., Shih, S.L., Lee, L.M. & Green, S.K. (2008). First report and molecular characterization of DNA A of three distinct begomoviruses associated with tomato leaf curl disease in Ghana. *Plant Disease* 92(11), 1585-1585.

Padidam, M., Beachy, R.N. & Fauquet, C.M. (1995). Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not essential for infectivity. *Journal of General Virology* 76, 25-35.

Pandey, P., Choudhury, N. & Mukherjee, S. (2009). A geminiviral amplicon (VA) derived from Tomato leaf curl virus (ToLCV) can replicate in a wide variety of plant species and also acts as a VIGS vector. *Virology Journal* 6(1), 152.

Pant, V., Gupta, D., Choudhury, N.R., Malathi, V.G., Varma, A. & Mukherjee, S.K. (2001). Molecular characterization of the Rep protein of the blackgram isolate of Indian mungbean yellow mosic virus. *Journal of General Virology* 82, 2559-2567.

Paprotka, T., Metzler, V. & Jeske, H. (2010). The first DNA 1-like α satellites in association with New World begomoviruses in natural infections. *Virology* 404(2), 148-157.

Pasumarthy, K.K., Mukherjee, S.K. & Choudhury, N.R. (2011). The presence of tomato leaf curl Kerala virus AC3 protein enhances viral DNA replication

and modulates virus induced gene-silencing mechanism in tomato plants. *Virology Journal* 8, 178.

Patil, B.L. & Fauquet, C.M. (2010). Differential interaction between cassava mosaic geminiviruses and geminivirus satellites. *Journal of General Virology* 91(Pt 7), 1871-82.

Pedersen, T.J. & Hanley-Bowdoin, L. (1994). Molecular characterization of the AL3 protein encoded by a bipartite geminivirus. *Virology* 202, 1070-1075.

Perring, T.M., Cooper, A.D., Rodriguez, R.J., Farrar, C.A. & Bellows, T.S. (1993). Identification of a whitefly species by genomic and behavioral studies. *Science* 259, 74-77.

Pieterse, C.M.J. & Dicke, M. (2007). Plant interactions with microbes and insects: from molecular mechanisms to ecology. *Trends in Plant Science* 12(12), 564-569.

Pita, J.S., Fondong, V.N., Sangre, A., Otim-Nape, G.W., Ogwal, S. & Fauquet, C.M. (2001). Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. *Journal of General Virology* 82, 655-665.

Prasanna, H.C. & Rai, M. (2007). Detection and frequency of recombination in tomato-infecting begomoviruses of South and Southeast Asia. *Virology Journal* 4, 111.

Prasanna, H.C., Sinha, D.P., Verma, A., Singh, M., Singh, B., Rai, M. & Martin, D.P. (2010). The population genomics of begomoviruses: global scale population structure and gene flow. *Virology Journal* 7, 220.

Priyadarshini, C.G.P., Ambika, M.V., Tippeswamy, R. & Savithri, H.S. (2011). Functional characterization of coat protein and V2 involved in cell to cell movement of cotton leaf curl Kokhran Virus-Dabawali. *PLoS ONE* 6(11).

Qazi, J., Amin, I., Mansoor, S., Iqbal, J. & Briddon, R.W. (2007). Contribution of the satellite encoded gene βC1 to cotton leaf curl disease symptoms. *Virus Research* 128(1-2), 135-139.

Qing, L. & Zhou, X. (2009). Trans-replication of, and competition between, DNA β satellites in plants inoculated with Tomato yellow leaf curl China virus and Tobacco curly shoot virus. *Phytopathology* 99(6), 716-720.

Raja, P. (2010). *Methylation of Geminivirus Genomes: Investigating its role as a host defense and evaluating its efficacy as a model to study chromatin methylation in plants.* Diss.:Ohio State University.

Raja, P., Wolf, J.N. & Bisaro, D.M. (2010). RNA silencing directed against geminiviruses: post-transcriptional and epigenetic components. *Biochimica et Biophysica Acta* 1799(3-4), 337-51.

Ratcliff, F., Harrison, B.D. & Baulcombe, D.C. (1997). A similarity between viral defense and gene silencing in plants. *Science* 276, 1558-1560.

Rigden, J.E., Dry, I.B., Mullineaux, P.M. & Rezaian, M.A. (1993). Mutagenesis of the virion-sense open reading frames of tomato leaf curl virus. *Virology* 193, 1001-1005.

Rojas, M.R., Hagen, C., Lucas, W.J. & Gilbertson, R.L. (2005). Exploiting chinks in the plant's armor: Evolution and emergence of geminiviruses. *Annual Review of Phytopathology* 43, 361-394.

Romay, G., Chirinos, D., Geraud-Pouey, F. & Desbiez, C. (2010). Association of an atypical alphasatellite with a bipartite New World begomovirus. *Archives of Virology* 155(11), 1843-1847.

Roossinck, M.J. (2011). The good viruses: viral mutualistic symbioses. *Nature Reviews. Microbiology* 9(February 2011), 99-108.

Rybicki, E.P. (1994). A phylogenetic and evolutionary justification for three genera of Geminiviridae. *Archives of Virology* 139, 49-77.

Saeed, S., Y. Zafar, Y., Randles, J.W. & Rezaian, M.A. (2007). A monopartite begomovirus-associated DNA β satellite substitutes for the DNA B of a bipartite begomovirus to permit systemic infection *Journal of General Virology* 88(10), 2881-2889.

Sakata, J.J., Shibuya, Y., Sharma, P. & Ikegami, M. (2008). Strains of a new bipartite begomovirus, pepper yellow leaf curl Indonesia virus, in leaf-curl-diseased tomato and yellow-vein-diseased ageratum in Indonesia. *Archives of Virology* 153(12), 2307-13.

Sánchez-Durán, M.A., Dallas, M.B., Ascencio-Ibañez, J.T., Reyes, M.I., Arroyo-Mateos, M., Ruiz-Albert, J., Hanley-Bowdoin, L. & Bejarano, E.R. (2011). Interaction between geminivirus replication protein and the sumo-conjugating enzyme is required for viral infection. *Journal of Virology* 85(19), 9789-9800.

Sanderfoot, A.A., Ingham, D.J. & Lazarowitz, S.C. (1996). A viral movement protein as a nuclear shuttle. *Plant Physiology* 11, 22-33.

Sanderfoot, A.A. & Lazarowitz, S.G. (1996). Getting it together in plant virus movement: cooperative interactions between bipartite geminivirus movement proteins. *Trends in Cell Biology* 6(9), 353-358.

Sanz, A.I., Fraile, A., Gallego, J.M., Malpica, J.M. & Garía-Arenal, F. (1999). Genetic variability of natural populations of cotton leaf curl geminivirus, single-stranded DNA virus. *Journal of Molecular Evolution* 49, 672-681.

Saunders, K., Bedford, I.D., Briddon, R.W., Markham, P.G., Wong, S.M. & Stanley, J. (2000). A unique virus complex causes *Ageratum* yellow vein disease. *Proceedings of the National Academy of Sciences USA* 97(12), 6890-6895.

Saunders, K., Bedford, I.D. & Stanley, J. (2001). Pathogenicity of a natural recombinant associated with Ageratum yellow vein disease: implications for geminivirus evolution and disease aetiology. *Virology* 282, 38-47.

Saunders, K., Briddon, R.W. & Stanley, J. (2008). Replication promiscuity of DNA-β satellites associated with monopartite begomoviruses; deletion mutagenesis of the Ageratum yellow vein virus DNA-β satellite localises sequences involved in replication. *Journal of General Virology* 89(12), 3165-3172.

Saunders, K., Lucy, A. & Stanley, J. (1991). DNA forms of the geminivirus African cassava mosaic virus consistent with a rolling circle mechanism of replication. *Nucleic Acids Research* 19, 2325-2330.

Saunders, K., Norman, A., Gucciardo, S. & Stanley, J. (2004). The DNA β satellite component associated with ageratum yellow vein disease encodes an essential pathogenicity protein (βC1). *Virology* 324, 37-47.

Saunders, K., Salim, N., Mali, V.R., Malathi, V.G., Briddon, R.W., Markham, P.G. & Stanley, J. (2002). Characterisation of Sri Lankan cassava mosaic virus and Indian cassava mosaic virus: evidence for acquisition of a DNA B component by a monopartite begomovirus. *Virology* 293(1), 63-74.

Saunders, K. & Stanley, J. (1999). A nanovirus-like component associated with yellow vein disease of *Ageratum conyzoides*: evidence for interfamilial recombination between plant DNA viruses. *Virology* 264, 142-152.

Schnippenkoetter, W.H., Martin, D.P., Willment, J.A. & Rybicki, E.P. (2001). Forced recombination between distinct strains of *Maize streak virus*. *Journal of General Virology* 82, 3081-3090.

Scholthof, K.B.G., Adkins, S., Czosnek, H., Palukaitis, P., Jacquot, E., Hohn, T., Hohn, B., Saunders, K., Candresse, T., Ahlquist, P., Hemenway, C. & Foster, G.D. (2011). Top 10 plant viruses in molecular plant pathology. *Molecular Plant Pathology* 12(9), 938-954.

Seal, S.E., van den Bosch, F. & Jeger, M.J. (2006). Factors influencing begomovirus evolution and their increasing global significance: implications for sustainable control. *Critical Reviews in Plant Sciences* 25, 23-46.

Selth, L.A., Dogra, S.C., Rasheed, M.S., Healy, H., Randles, J.W. & Rezaian, M.A. (2005). A NAC domain protein interacts with tomato leaf curl virus replication accessory protein and enhances viral replication. *Plant Cell* 17(1), 311-25.

Settlage, S.B., See, R.G. & Hanley-Bowdoin, L. (2005). Geminivirus C3 protein: replication enhancement and protein interactions. *Journal of Virology* 79, 9885-9895.

Sharma, P., Gaur, R.K. & Ikegami, M. (2011). Subcellular localization of V2 protein of *Tomato leaf curl Java virus* by using green fluorescent protein and yeast hybrid system. *Protoplasma* 248(2), 281-8.

Sharma, P. & Ikegami, M. (2009). Characterization of signals that dictate nuclear/nucleolar and cytoplasmic shuttling of the capsid protein of Tomato leaf curl Java virus associated with DNAβ satellite. *Virus Research* 144(1-2), 145-153.

Shih, S., Kumar, S., Tsai, W., Lee, L. & Green, S. (2009). Complete nucleotide sequences of okra isolates of Cotton leaf curl Gezira virus and their associated DNA-β from Niger. *Archives of Virology* 154(2), 369-372.

Shimura, H. & Pantaleo, V. (2011). Viral induction and suppression of RNA silencing in plants. *Biochimica et Biophysica Acta* 1809(11-12), 601-612.

Singh, D.K., Malik, P.S., Choudhury, N.R. & Mukherjee, S.K. (2008). MYMIV replication initiator protein (Rep): Roles at the initiation and elongation steps of MYMIV DNA replication. *Virology* 380(1), 75-83.

Stanley, J., Saunders, K., Pinner, M.S. & Wong, S.M. (1997). Novel defective interfering DNAs associated with Ageratum yellow vein geminivirus infection of *Ageratum conyzoides*. *Virology* 239, 87-96.

Sukamto, Kon, T., Hidayat, S.H., Ito, K., Hase, S., Takahashi & Ikegami, M. (2005). Begomoviruses associated with leaf curl disease of tomato in Java, Indonesia. *Journal of Phytopathology* 9, 562-566.

Swanson, M.M. & Harrison, B.D. (1993). Serological relationships and epitope profiles of isolates of okra leaf curl geminivirus from Africa and the Middle East. *Biochimie* 75, 707-711.

Tahir, M.N., Amin, I., Briddon, R.W. & Mansoor, S. (2011). The merging of two dynasties-identification of an African cotton leaf curl disease-associated begomovirus with cotton in Pakistan. *PLoS ONE* 6(5), e20366.

Tao, X. & Zhou, X. (2008). Pathogenicity of a naturally occurring recombinant DNA satellite associated with tomato yellow leaf curl China virus. *Journal of General Virology* 89(1), 306-311.

Thresh, J.M. & Cooter, R.J. (2005). Strategies for controlling cassava mosaic virus disease in Africa. *Plant Pathology* 54, 587-614.

Tiendrébéogo, F., Lefeuvre, P., Hoareau, M., Traoré, V.S.E., Barro, N., Reynaud, B., Traoré, A.S., Konaté, G., Traoré, O. & Lett, J.-M. (2009). Occurrence of *East African cassava mosaic virus*-Uganda (EACMV-UG) in Burkina Faso. *Plant Pathology* 58(4), 783-783.

Tiendrébéogo, F., Lefeuvre, P., Hoareau, M., Villemot, J., Konate, G., Traore, A.S., Barro, N., Traoré, V.S., Reynaud, B., Traoré, O. & Lett, J.M. (2010). Molecular diversity of Cotton leaf curl Gezira virus isolates and their satellite DNAs associated with okra leaf curl disease in Burkina Faso. *Virology Journal* 7(1), 48.

Tomas, D.M., Canizares, M.C., Abad, J., Fernandez-Munoz, R. & Moriones, E. (2011). Resistance to Tomato yellow leaf curl virus accumulation in the tomato wild relative Solanum habrochaites associated with the C4 viral protein. *Mol Plant Microbe Interact* 24(7), 849-61.

Trinks, D., Rajeswaran, R., Shivaprasad, P.V., Akbergenov, R., Oakeley, E.J., Veluthambi, K., Hohn, T. & Pooggin, M.M. (2005). Suppression of RNA silencing by a geminivirus nuclear protein, AC2, correlates with transactivation of host genes. *Journal of Virology* 79(4), 2517-2527.

Urbino, C., Polston, J.E., Patte, C.P. & Caruana, M.-L. (2003). Characterization and genetic diversity of Potato yellow mosaic virus from the Caribbean. *Archives of Virology* 149, 417 - 424.

Van Den Bosch, F., Akudibilah, G., Seal, S. & Jeger, M. (2006). Host resistance and the evolutionary response of plant viruses. *Journal of Applied Ecology* 43(3), 506-516.

van der Walt, E., Martin, D.P., Varsani, A., Polston, J.E. & Rybicki, E.P. (2008). Experimental observations of rapid Maize streak virus evolution reveal a strand-specific nucleotide substitution bias. *Virology Journal* 5, 104.

Van Regenmortel, M.H.V. (2006). Virologists, taxonomy and the demands of logic. *Archives of Virology* 151(7), 1251-1255.

Vanitharani, R., Chellappan, P. & Fauquet, C.M. (2005). Geminiviruses and RNA silencing. *Trends in Plant Science* 10, 144-151.

Vanitharani, R., Chellappan, P., Pita, J.S. & Fauquet, C.M. (2004). Differential roles of AC2 and AC4 of cassava geminiviruses in mediating synergism and suppression of posttranscriptional gene silencing. *Journal of Virology* 78(17), 9487-9498.

Varma, A. & Malathi, V.G. (2003). Emerging geminivirus problems: A serious threat to crop production. *Annals of Applied Biology* 142(2), 145-164.

Venkataravanappa, V., Lakshminarayana Reddy, C., Swaranalatha, P., Jalali, S., Briddon, R.W. & Reddy, M.K. (2011). Diversity and phylogeography of begomovirus-associated beta satellites of okra in India. *Virology Journal* 8, 555.

Wege, C., Gotthardt, R.-D., Frischmuth, T. & Jeske, H. (2000). Fulfilling Koch's postulates for *Abutilon mosaic virus*. *Archives of Virology* 145, 2217-2225.

Welker, N.C., Habig, J.W. & Bass, B.L. (2007). Genes misregulated in C. elegans deficient in Dicer, RDE-4, or RDE-1 are enriched for innate immunity genes. *RNA* 13(7), 1090-1102.

Wyatt, S.D. & Brown, J.K. (1996). Detection of Subgroup III Geminivirus Isolates in Leaf Extracts by Degenerate Primers and Polymerase Chain Reaction. *Phytopathology* 86(12), 1288-1293.

Xie, Q., Suárez-López, P. & Gutiérrez, C. (1995). Identification and analysis of a retinoblastoma binding motif in the replication protein of a plant DNA virus: requirement for efficient viral DNA replication. *EMBO Journal* 14, 4073-4082.

Xie, Y., Wu, P., Liu, P., Gong, H. & Zhou, X. (2010). Characterization of alphasatellites associated with monopartite begomovirus/betasatellite complexes in Yunnan, China. *Virology Journal* 7(1), 178.

Yadava, P., Suyal, G. & Mukherjee, S.K. (2010). Begomovirus DNA replication and pathogenicity. *Current Science* 98(3), 360-368.

Yang, X., Baliji, S., Buchmann, R.C., Wang, H., Lindbo, J.A., Sunter, G. & Bisaro, D.M. (2007). Functional modulation of the geminivirus AL2 transcription factor and silencing suppressor by self-interaction. *Journal of Virology* 81(21), 11972-11981.

Zaffalon, V., Mukherjee, S.K., Reddy, V.S., Thompson, J.R. & Tepfer, M. (2011). A survey of geminiviruses and associated satellite DNAs in the cotton-growing areas of northwestern India. *Archives of Virology*, 1-13.

Zhang, S.C. & Ling, K.S. (2011). Genetic diversity of sweet potato begomoviruses in the United States and identification of a natural recombinant between sweet potato leaf curl virus and sweet potato leaf curl Georgia virus. *Archives of Virology* 156(6), 955-968.

Zhang, T., Luan, J.B., Qi, J.F., Huang, C.J., Li, M., Zhou, X.P. & Liu, S.S. (2012). Begomovirus-whitefly mutualism is achieved through repression of plant defences by a virus pathogenicity factor. *Molecular Ecology* 21(5), 1294-304.

Zhou, X., Liu, Y., Robinson, D.J. & Harrison, B.D. (1998). Four DNA-A variants among Pakistani isolates of cotton leaf curl virus and their affinities to DNA-A of geminivirus isolates from okra. *Journal of General Virology* 79, 915-923.

Zhou, Y.C., Noussourou, M., Kon, T., Rojas, M., Jiang, H., Chen, L.F., Gamby, K., Foster, R. & Gilbertson, R. (2008). Evidence of local evolution of tomato-infecting begomovirus species in West Africa: characterization of tomato leaf curl Mali virus and tomato yellow leaf crumple virus from Mali. *Archives of Virology* 153(4), 693-706.

Zrachya, A., Glick, E., Levy, Y., Arazi, T., Citovsky, V. & Gafni, Y. (2007). Suppressor of RNA silencing encoded by *Tomato yellow leaf curl virus*-Israel. *Virology* 358, 159-165.

Zulfiqar, A., Zhang, J., Cui, X., Qian, Y., Zhou, X. & Xie, Y. (2012). A new begomovirus associated with alpha- and betasatellite molecules isolated from Vernonia cinerea in China. *Archives of Virology* 157(1), 189-191.

Acknowledgements

All glories & loves be to **Allah (SW)** and His Prophet **Muhammad (SAW).**

I cannot say a single word of THANKS for my **Father** and my **Mother**. To me there is no word discovered so far to say thanks to you. I just say I LOVE YOU BOTH!

I want to thank my whole family in Pakistan especially **my brothers** and my beloved **sister**, whose love and care always inspire me. Thank you very much for always motivate me to achieve big aims.

My supervisor, **Dr. Anders Kvarnheden**, you will always be a sign of inspiration for me in science. To me you were not a boss, you were my spiritual father. Thanks for taking care of me.

My co-supervisor in Pakistan, **Dr. Muhammad Saeed**, you taught me the first lesson in practical science-How To Use A Pippete. You were always amazing and whenever I need guidance you were with me.

My Co-supervisor in SLU, **Dr. Eugene Savenkov**, Thank you very much for your innovative ideas and your guidance whenever I need.

The first person in Sweden whom I know after Dr. Anders, **Dr. Jon Ramsell**. Thank you very much for your help and care. You lead me to Gottsunda through the shortest way, which I am still using.

I would like to thank in a special way to **Dr. Rob W. Briddon** in NIBGE, Pakistan-a legendary personality in Plant Virology.

The Higher Education Commission (**HEC**) Pakistan for giving me the opportunity to explore this entirely new world, Swedish Institute (**SI**) for taking care of my scholarship and **Magnus Bergwalls' Foundation** for the financial support for my project.

I always miss my friends in Pakistan, **Akhlaq**, **Haroon**, **Zulqarnain**, **Shahzad Anjum**, **Nouman**, **Mubin**, **Mehmood**, **Furrukh**, **Shafiq** and **Naeem**. We always enjoyed life together, laugh together and had much more fun. I am always proud to be your friend.

I want to thank a very special friend **Dr. Shahnawaz** who was more like a brother to me.

My **lab family** was awesome and everyone has his/her own special place in my life here in VBSG. **Ingela**, **Nadeem**, **Ulrike**, **Nina**, **Jim**, **Walter**, **Kadri**, thanks for being with me in the lab.

Special words for **Ingrid Ericsson**, **Mona Munther**, **Birgitta, Gunilla, Urban** and all technical staff in VBSG, who made my life easy. Especially Ingrid and Mona! You GIRLS were really loving and so kind that I can never forget you. You always remind me the love and care of my mother.

Dr. Björn Nicander thank you very much for keeping my computer in running condition (although it was an old buddy).

Dr. Lars & **Eva Lars** for making my life easy when I was new in Sweden.

My friend **Ramesh Vetukuri** you were always there whenever I had some problem. **Laura Vetukuri** of course, you are simply amazing.

How can I forget Innebandy with such good people (**Kjell**, **Usman**, **Tom**, **Philip**, **Vestman**, **Uddenberg**, **Abdul Ghafoor**, **Arne**, **Eric** and everyone else). We had great fun together on every Tuesday and Thursday.

Dr. Sarosh Bejai thank you very much for your help and guidance whenever I need. Please visit me in Pakistan to see "SOLO CHICKEN SOUP".

Special thanks to **David Kradolfer** for calling me exactly at 16:00 every day to play table tennis-you were really a great rival of mine.

Shahid we had very good time together in Sweden, playing cricket and enjoying McDonald. Thank you very much.

Sweden blessed me some sweet friends. **Mazhar**, **Mohsin**, **Shehzad**, **Mubashar**, **Shahid Abro**, **Allah Rakha**, **Munir**, **Khurrum**, **Shah**, **Akhtar**, **Shashi** and everyone whom I couldn't mention, you were all great to me. Especially **Mazhar** and **Mohsin** you cooked food for me when I need your help during my write up. What a taste you have in your hands! YUMMY.

If you really want to enjoy your PhD studies you must experience with master students and lab trainees. I am thankful to my master students **Sunil**, **Lars**, **Maaike**, **Mi Zhou**, **Elham**, **Sowmya**, **Indu** and others for giving me such a nice experience and much fun.

Some people have very special impact on our lives. I want to say thanks to all those who touched my life in one way or another.

MUHAMMAD NAEEM SATTAR