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Current Status of *Geminivirus* in India: RNAi Technology, A Challenging Cure

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ABSTRACT

Geminivirus are a large diverse family of plant viruses that infect a broad variety of plants and cause significant loss to Agricultural crops and ornamental plants in India. The vector white fly is the carrier of these viruses. This review focuses on the current status of *Geminivirus* in India and research related to it. Researches being carried out on *Geminivirus* include their molecular characterization, serological and computational characterization. Many costly measures in controlling the infections and increasing the crop yield have been applied. A cost effective appraise against these viruses is the relevance of RNAi Technology. RNAi is a challenging approach in which an interference RNA molecule put a check halt on the multiplication of *Begomovirus* in plants through its ability to form a Dicer complex.

Key words: *Geminivirus*, agricultural, India, white fly, RNAi, *Begomovirus*, dicer

INTRODUCTION

India has a long history of agriculture farming. Ancient records proved that agriculture is far long being carried out since ages, almost 9000 BC. India is present a developing country. But that day is not so far when India will be a developed country. In making India a developed country maximum contribution to its economy comes from the agriculture sector. According to ICAR Annual Report 2011-2012, in India the growth of agriculture and improving income and livelihood of small holders in agriculture constitute more than 80% of the total farming households, 50% of rural households and 36% of total households in India. Principally agriculture practices in India are seasonal. But with the release of more and more hybrid varieties, crop plants are cultivated through out the year. Different types of food crops are grown in India such as cereals, pulses, vegetables and fruits (Bhattacharyya and Chakraborty, 2005).

A nearly developed country like India ranking second in farm output worldwide has its major contribution to its economy comes from the agriculture sector. A large number of crop plants are cultivated in India since ages and some of them are unique in their own way. Many have medicinal properties either (Meetei *et al.*, 2012). There are a large number of threats to the agricultural crops grown not in India itself but even across the worldwide (AVRDC, 2012). Large number of pest harms the crops and hinders the production yield (Kataria and Kumar, 2012). But apart from them there are also other microscopic elements that cause a great annual loss to the agriculture sector thus in turn harming the livelihood of the farmers of India and Indian economy. This none other microscopic elements are viruses (CRSP, 2012). Several viruses affect the

crop plants, but the major contribution is from the viruses belonging to the Geminiviridae family. These viruses are transmitted from plant to plant by their main insect host well-known as white fly (*Bemisia tabaci*) (Wang *et al.*, 2012). Other insects such as leafhopper and treehopper are also a causal agent.

This review article depicts the current status of *Geminivirus* in India highlighting various research institute and university where work on Geminiviruses are being carried out and includes a wide range of information mentioning the new disease reports from throughout India. Moreover a list of various Geminiviruses species in India has also being mentioned which gives an idea of new viruses introduced in India and evolution of existing viruses through recombination. To find a cure against theses Geminiviruses RNAi technology (Vanitharani *et al.*, 2003; Kurth *et al.*, 2012) is a wonderful tool which employs a check on the devastating nature of Geminiviruses infecting ornamental plants, weeds and crops. Results of these techniques effectively applied for disease management and development of quarantine strategies for handling and transportation of infected plant samples. Thus, it's an approach to bring the research work of various scientists working on Geminiviruses in India at one place in this review article.

GEMINIVIRIDAE FAMILY AND ITS TRANSMISSION VECTORS

In plants 47% infections are caused by viruses. Geminiviridae is the family which consists of maximum number of viruses (Fauquet *et al.*, 2005). General symptoms of diseases caused by *Geminivirus* are curling of leaves, yellowing of veins, yellow mosaic patterns, dwarfing of leaves. Viruses belonging to Geminiviridae family are plant viruses which are obligate intracellular parasites, having no self machinery to replicate themselves (Stanley *et al.*, 2005). It comprises of four genera: *Begomovirus*, *Curtovirus*, *Mastrevirus* and *Topocuvirus*. *Begomovirus* is one the biggest genera of the family (Medina-Ramos *et al.*, 2008). It comprises of around 200 species existing worldwide. *Begomovirus* principally affect the dicotyledonous plant species. These viruses are transmitted by whitefly (Markham *et al.*, 1994).

White fly (*Bemisia tabaci*) belongs to Aleyrodidae family of class Insecta. This fly prevails more in the tropical and subtropical regions of the globe (Sidhu *et al.*, 2009). *Begomovirus* genera comprises of most multifarious genome among other genera's. It has a bipartite genome having two circular DNA designated as DNA A and DNA B. Both DNA A and DNA B range from 2.7 to 3 kb (Hanley-Bowdoin *et al.*, 1999). DNA B is dependent on DNA A for its doings. Moreover in up to date publication an additional circular DNA has been reported as ß satellite, also contribute to *Begomovirus* virulation (Briddon *et al.*, 2001) and nanovirus-like DNA satellite molecules (alphasatellites) (Briddon and Stanley, 2006).

Curtovirus another genera of the family consist of 7 species reported so far. The species are Beet curly top Iran virus, Beet curly top virus, Beet mild curly top virus, Beet severe curly top virus, Horseradish curly top virus, Pepper curly top virus and Spinach curly top virus (Bolok Yazdi *et al.*, 2008). *Curtovirus* are transmitted through leaf hopper or treehopper (*Micrutalis malleifera*). They have a monopartite genome. The genome comprises of a circular single stranded DNA molecule ranges up to 3 kb (Baliji *et al.*, 2004). *Mastrevirus* is the second largest genera of this family. It comprises of 14 species as given in ICTV up to date (Brown *et al.*, 2012). Infection of *Mastrevirus* is caused by leaf hopper which is its chiefly host. Leaf hopper belongs to Cicadellidae family. Leaf hopper is a common name applied to any species of *Cicadellidae* family (Lee *et al.*, 2000). Leaf hopper exists all over the world and do not have a pupae stage while turning into adult from nymph. *Mastrevirus* affect both dicots and monocots plants species. Genome is monopartite and size is up to 2.7 kb. *Topocuvirus* is the smallest genera of Geminiviridae family (Briddon and Markham, 2001). It consists of only one member species reported in the world, known as Tomato

pseudo-curly top virus. It infects the dicotyledonous plant, mainly tomato. It has a monopartite, closed circular, single stranded DNA, about 2.8 kb nucleotides long. The virus is transmitted by treehopper (Hunter *et al.*, 1998).

GENOME ORGANIZATION

According to the below given diagram (Fig. 1) the two circular genome of the *Begomovirus* can be understood easily. DNA A has 6 Open Reading Frames (ORF). They are *AC1, AC2, AC3, AC4, AV1* and *AV2*. *AC1 (AL1)* encodes for Replication initiation protein (Rep) (Saunders *et al.,* 2008). *AC2 (AL2)* produces Transcription activator protein (TrAP). *AC3 (AL3)* encodes for replication enhancer proteins (Tiendrebeogo *et al.*, 2008).

AC4 functions for determining symptoms expression. *AV1* (*AR1*) produces Coat Protein (CP). *AV2* is the movement protein also called as ("precoat" ORF). DNA B has two ORF. *BV1* (*BR1*) is the producer of Nuclear shuttle protein (NSP). *BC1* (*BL1*) instructs for Movement protein for cell to cell transfer (Rojas *et al.,* 2005). *AV1, AV2* and *BV1* are the plus (+) virion sense strand, whereas, *AC1, AC2, AC3, AC4* and *BC1* represents for negative (-) complementary sense strand (Yadava *et al.*, 2010). According to Fig. 2, *Curtovirus* has seven ORFs (Park *et al.*, 1999). Out of them three open reading frames (ORFs) are in the positive (virion) sense i.e., *V1, V2, V3* and the rest four in the negative (complementary) sense i.e., *C1, C2, C3* and *C4*. *V1* encodes for coat protein, *V2* produces

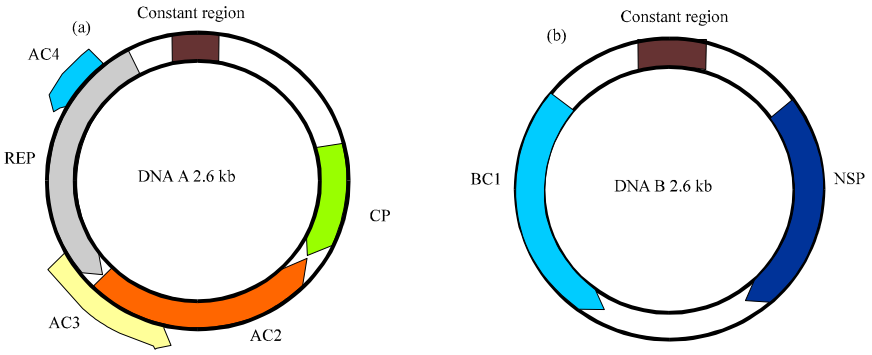

Fig. 1 (a-b): *Begomovirus* genome components, CP: Coat protein, Rep: Replication-associated protein, NSP: Nuclear shuttle protein

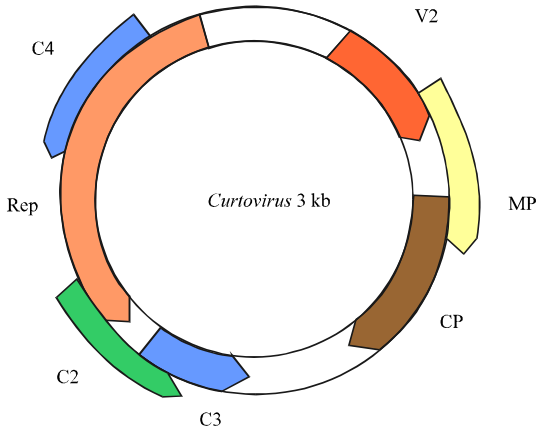

Fig. 2: *Curtovirus* genome components, CP: Coat protein, Rep: Replication-associated protein, MP: Movement protein

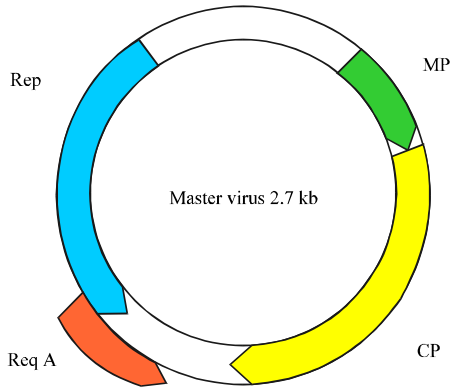

Fig. 3: *Mastrevirus* genome components, CP: Coat protein, Rep: Replication-associated protein, MP: Movement protein

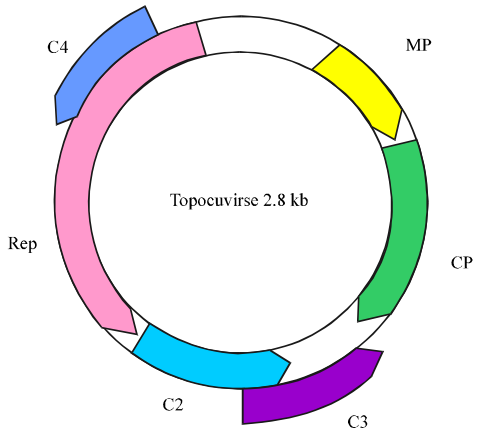

Fig. 4: *Topocuvirus* genome components, CP: Coat protein, Rep: Replication-associated protein, MP: Movement protein

an ss/dsDNA regulator, *V3* instruct for movement protein. *C1* is assigned for replication protein whereas, the *C2* has unknown function. *C3* is for the production of protein which is similar to the replication enhancer protein of *Begomovirus*. *C4* initiates cell division and for symptom determinant (Park *et al*., 2003).

As per Fig. 3 there are four ORFs in the *Mastrevirus* genome (Boulton, 2002). Two of them on the virus (+) sense and rest two on the complementary (-) senses (Heyraud-Nitschke *et al*., 1995). *V1* is the larger plus (+) sense ORF which encodes for the coat protein (CP) (Gutierrez *et al*., 2004). *V2* produces Cell-to-cell movement protein (Nahid *et al*., 2008). Complementary sense has two components, *C1* encodes for Rep A replication-associated protein having and *C1:C2* instruct for Replication-associated (Rep) protein which is expressed from ORFs *C1* and *C2* by transcript splicing (Xie *et al*., 1999). *Topocuvirus* genome in the Fig. 4 have six ORFs. *V1* and *V2* instruct for coat protein and movement protein respectively (Varma and Malathi, 2003). Replicase A and Replicase B are respectively produced by *C1* and *C2*. *C3* encodes for replication enhancer protein having function similar to that of *Begomovirus* rephication enhancer protein. Cell division is initiated by the *C4* component. *V1* and *V2* fall in the plus sense and *C1*, *C2*, *C3* comes in the negative sense region (Govindappa *et al*., 2011).

REPLICATION: A GENERAL VIEW

Geminiviruses of the genera *Begomovirus* and *Curtovirus* utilize three replication modes: Complementary-Strand Replication (CSR), Rolling-Circle Replication (RCR) (Saunders *et al.*, 1991) and Recombination-Dependent Replication (RDR) (Alberter *et al.*, 2005). But RCR is the general mechanism common to all the four genera (Fig. 5). It is not possible for the Geminiviruses to replicate themselves (Bisaro, 1996). So, they evolve themselves by using plant cell machinery for their survival. They undergo rolling circle replication through which their DNA replicates. This process is quite similar to the infection of Bacteriophages like M13. Principally these viruses depend on host DNA Polymerases, probably repair polymerases (Chasan, 1995). Moreover, transcription factors are also the main ingredients in their multiplication.

The first step in their proceedings in the entry of the viruses in the plant cells which is carried out by their transmission vectors. Once they are inside as soon as possible their genomic DNA gets in the cell nucleus of the infected host cell to assist with viral DNA replication. Rep commences virus-specific recognition of its cognate origin (Hanley-Bowdoin *et al.*, 1999). Their single stranded circular DNA gets converted into a double stranded DNA. Their ssDNA is called as a plus or + or positive sense strand acting as a template DNA. The complementary DNA which is formed from the template DNA using DNA repair enzymes is called as a negative (-) sense strand (Kaliappan *et al.*, 2012). Now the Rep protein of the virus cleaved the viral strand at their specific origin of replication site, thus the rolling circle phase starts. Now the new single stranded DNA gets packed into the geminate particles in the nucleus and is ready for new infections (Gutierrez, 2000).

CURRENT RESEARCH ON GEMINIVIRUSES

This review article illustrates the existing status of *Geminivirus* in India. It enlightens the research being carried out on *Geminivirus* which includes their molecular, serological and computational characterization. Moreover, stating various research institute and university where works on geminiviruses are being carried out and includes a wide range of information mentioning the new disease reports from throughout India.

A variant of tomato leaf curl Bangladesh virus was found infecting *Gaillardia* (Mahatma and Mahatma, 2012). Sequence analysis using the BLAST programme revealed nucleotide sequence identities of 94.0% with Tomato leaf curl Bangladesh virus-(Bangladesh:2) (ToLCBDV-[BD:2]) (AF188481) and 92.0% with Chilli leaf curl virus-DU [India: New Delhi: Papaya: 2009 (HM140364). Moreover a *Begomovirus* infecting *Mentha spicata* was also reported in Lucknow and in the Tarai region of Uttaranchal province. Its sequence showed 93% identity with the *Begomovirus* Tomato leaf curl Pakistan virus (Samad *et al.*, 2009). This confirms the invasion of foreign species in India from adjacent countries.

Sunn hemp (*Crotalaria juncea*) extensively cultivated in India as a fiber or green manure crop which was found infected by an Indian tomato leaf curl virus. The CP gene of Indian tomato leaf curl virus was used as a DNA probes (IToLCV) for the identification of the disease (Khan *et al.*, 2002). *Cyamopsis tetragonoloba (*Guar*)*, an important cultivated crop in India was found infected by Tomato leaf curl virus (ToLCV). Nucleotide sequence identity regions of the *CP* genes of Tomato leaf curl virus (ToLCV) of Indian isolates were carried out (Khan *et al.*, 2003). It revealed mutual

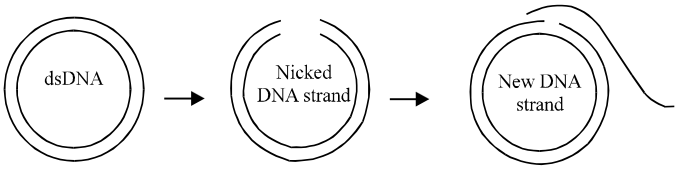

Fig. 5: Rolling circle replication

95% sequence identity with ToLCV-BanII (accession number U38239); 91% with ToLCV-BanI (accession no. Z 48182), ToLCV-BaV (accession no. AF 295401) and ToLCV-Kolar (accession no. AF 428255); 90% with ToLCV-BanIV (accession no. AF 165098). *Glycine max* (soybean) was also affected by *Begomovirus*. When analysis of nucleotide sequence was done by BLAST the search revealed that the highest nucleotide sequence identity (96-98%) comes with Tomato leaf curl Karnataka virus (ToLCKV) (TLU38239, AJ810342, AY753203, AJ810370 and AJ810347) (Raj *et al.*, 2006).

Sequence analysis of *Calendula officinalis* revealed the highest nucleotide sequence identities (95, 94 and 93%) of the virus infecting *Calendula* with Tobacco curly shoot virus (AF240675), *Ageratum* enation virus (AJ437618) and Tomato leaf curl Bangladesh virus (AF188481), respectively (Khan *et al.*, 2005). *Sechium edule* (Chayote) belongs to Cucurbitaceae vegetable family commonly grown in Darjeeling and Sikkim hills regions of India during the summer-rainy season. The maximum sequence identity (95%) was found with ToLCV-NDe from Pakistan (AF448058), but have only 70.7% identity with Chayote yellow mosaic virus (ChYMV; AJ223191), which is the uncharacterized virus, reported from Nigeria (Mandal *et al.*, 2004).

A new monopartite *Begomovirus* species, Chilli leaf curl Vellanad virus and associated betasatellites was found infecting chilli in the Vellanad region of Kerala, India. It was fully sequenced and revealed a viral genome (HM007121) of 2,788 nt consisting of seven predicted ORFs (AV1, AV2, AC1, AC2, AC3, AC4, AC5). Sequence alignment was done using MegAlign (DNASTAR, Madison, WI) and demonstrated that the virus had the highest levels of nucleotide sequence identity (77%) with a 'Pakistan" strain of Pepper leaf curl Bangladesh virus (AF314531) (Kumar *et al.*, 2012b). *Clerodendrum inerme* belongs to family Verbenaceae and it is a common hedge type plant used to grow in gardens. 80% nucleotide sequence identity matches with the Cotton leaf curl kokhran virus (AY456683). Les then 78% identity were detected against ToLCNDV (John *et al.*, 2006).

Leaf curl disease in potato (*Solanum tuberosum*) was found to be caused by a strain of Tomato leaf curl New Delhi virus (ToLCNDV). It had 93-95% sequence identity with the DNA A of ToLCNDV and have less then 75% identity with other Tomato leaf curl virus isolates and Potato yellow mosaic virus (Usharani *et al.*, 2004). *Begomovirus* affecting *Gossypium barbadense* depicted 84-85% nucleotide sequence identity of its DNA A with the Cotton leaf curl Multan virus (CLCuMV, AJ002447, AJ002459), followed by 83.4% with Malvastrum yellow vein virus (MYVV, AJ457824). On the other hand conserved C1 gene of the DNA-B showed highest nucleotide identities about 89.1-89.6% and amino acid sequence identity of about 80.5-81.4% with similar sequences from tomato (AJ316035) and *G. hirsutum* (AJ298903) reported from Pakistan (Reddy *et al.*, 2005).

Radish leaf curl virus was found infecting okra in Bihar state of India. For virus and satellite transmission from field-collected infected plants to healthy tobacco and okra, Healthy whiteflies (~25) were used for infectivity assay. Tobacco and okra (10 plants each) was inoculated with a mixture of *Begomovirus* and alpha- and betasatelhte infectious clones. Plants of both assays yielded typical symptoms of leaf curling and stunting identical to those observed previously in the field (Kumar *et al.*, 2012a). A *Begomovirus* caused Indian dolichos yellow mosaic disease in Dolichos (*Lablab purpureus*) which is a popular leguminous vegetable cultivated in India. It had 95.3 and 93.4% nucleotide sequence identity with DoYMV-[Bangladesh] (AY271891) and DoYMV originating from India (AY309241), respectively (Maruthi *et al.*, 2006). *Capsicum annuum* (Chilli) is cultivated throughout India as an important spice crop. The plant was found affected by Chilli leaf curl virus. It demonstrate closed similarity of its replication initiator protein, AV1 and AV2 genes of Chilli leaf curl virus-[Pakistan: Multan] (ChiLCuV-[Pk:Mul]; AF336806) (Senanayake *et al.*, 2007).

In another report Chilli was also affected by Tomato leaf curl New Delhi virus (ToLCNDV). It showed 89-93% identity with various ToLCNDV viruses like X78956, AY428769, TLU15016 and AF448058 (Khan *et al.,* 2005). *Vigna unguiculata* (Cowpea) were infected by Mungbean yellow mosaic India virus. The nucleotide sequence of its DNA **β** has shown 59% identity with DNA **β** of Cotton leaf curl Rajasthan virus (Accession No. AY083590) (Rouhibakhsh and Malathi, 2005). Tomato leaf curl Joydebpur virus was found infecting *Capsicum annuum*. The chilli *Begomovirus* genome organization from Punjab shows 90.8 and 90.3% sequence identity to that of tomato *Begomovirus* from India (DQ629103) and tomato leaf curl Joydebpur virus (ToLCJV, AJ875159), respectively (Shih *et al.*, 2007). A bipartite *Begomovirus* affecting tomato in India was found. The study demonstrates the role of a betasatellite in the pathogenesis of tomato leaf curl New Delhi virus (ToLCNDV). For infection, DNA A alone of ToLCNDV could infect tomato and *Nicotiana benthamiana* and induce mild symptoms, but DNA B or Cotton leaf curl Multan betasatellite (CLCuMB) was required for development of typical leaf curl symptoms (Sivalingam and Varma, 2012).

Yellow vein mosaic disease was found infecting *Abelmoschus esculentus* L., New Delhi, India. DNA-A and DNA-B was completely sequenced and were comprised of 2,746 and 2,703 nucleotides, respectively. The betasatellite (DNA-**β**) component was absent in the sample (Venkataravanappa *et al.*, 2012). Comparison of DNA-A component with other known begomoviruses showed 85.9% similarity with bhendi yellow vein mosaic Delhi virus [BYVDV-IN (India: Delhi: Okra)]. DNA-B showed highest sequence identity (87.8% identical) to that of a ToLCNDV (AY158080). *Cajanus cajan* (Pigeonpea) belongs to family Fabaceae is affected by *Begomovirus* (Acc. No. AY927997). When compared it confirmed highest nucleotide sequence identities of about 95-97% with ToLCNDV-Mild (U15016); ToLCNDV-[Lucknow] (Y16421); ToLCNDV-[Solanum] (AJ620187); a ToLCNDV isolated from Luffa (AY309957); *Cucurbita maxima* yellow mosaic virus (AY396151) and Pumpkin yellow vein mosaic virus (AY184487) (Raj *et al.*, 2005). *Hibiscus cannabinus* (Kenaf) belongs to family Malvaceae grown as a fiber crop in India. Its PCR was done by using forward and reverse primers. The forward primer has sequence 5'-CAGAAGCCCTGATGTTCCAAG-3' and the reverse primer has sequence 5'-TACATCCTGTACAGTCTGGGC-3' which was used against the coat protein gene of the *Begomovirus* (EU366903) (Paul *et al.*, 2009). The *Begomovirus* and betasatellite showed the highest levels of sequence identity (95 and 92%, respectively) with tomato leaf curl Joydebpur virus (ToLCJV, AJ875159) and its associated betasatellite (ToLCJB, AJ966244) reported from Bangladesh.

Cloning and sequencing were done to partially characterize the *Begomovirus* infecting *Sida cordifolia* L., *Croton bonplandianum* L., *Malachra capitata* L., *Eclipta prostrata* L., *Clerodendrum inerme* L., *Acalypha indica* L. and *Urena lobata* L. which are common weeds found all over India. In this study Coat Protein (CP) genes were found to be amplified from all the infected samples tested, whereas betasatellite molecules amplified only from four infected samples (Sida, Croton, Malachra and Urena). Their genetic pattern was compared with respective geographical isolates throughout India (Paul *et al.*, 2012). Jute (*Corchorus capsularis*) one of the largest fiber crops cultivated in India. It belongs to the family Tiliaceae. Found to be infected by *Begomovirus* (EU047706) showing Yellow mosaic disease. BLAST analysis revealed that it showed 91.2% similarity in its nucleotide sequence with Corchorus golden mosaic virus (DQ641688) (Ghosh *et al.*, 2008). *Begomovirus* (EU439259) affecting *Amaranthus cruentus* (Grain amaranth) and causing yellow vein net disease has shown 98% sequence identity with Ageratum Enation Virus (AEV) isolates (AJ437618) (Raj *et al.*, 2008). Current research on Geminiviruses (Fig. 6) are carried out at various places in renowned research institutions in India.

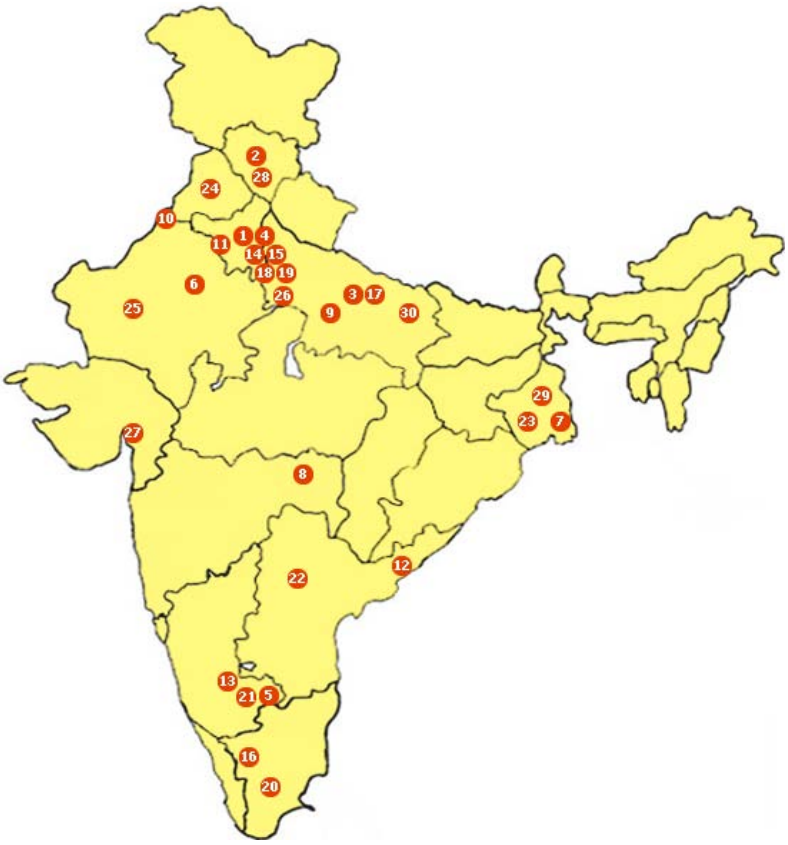

Fig. 6: *Geminivirus* research area in India, 1: International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi (http://www.icgeb.org/home.html), 2: Institute of Himalayan Bioresource and Technology (IHBT), Palampur, H.P. (http://www.ihbt.res.in/), 3: National Botanical Research Institute (NBRI), Lucknow, U.P. (http://www.nbri.res.in/), 4: University of Delhi (DU), New Delhi (http://www.du.ac.in/index.php?id=4http://www .iisc.ernet.in/), 5: Indian Institute of Science (IISc), Bangalore, Karnataka (http://www.iisc.ernet.in/), 6: Mody Institute of Technology and Science (MITS), Lakshmangarh, Rajasthan (http://www.mitsuniversity.ac.in/), 7: Central Research Institute for Jute and Allied Fibres (CRIJAF), Barrackpore, Kolkata, West Bengal (http://www.crijaf.org.in/), 8: Central Institute for Cotton and Research, (CICR), Nagpur, Maharashtra (http://www.cicr.org.in/ongoingpprojects.html), 9: Indian Institute of Pulses Research (IIPR), Kanpur, U.P. (http://www.iipr.res.in/), 10: Agricultural Research Station (ARS), Sriganganagar, Rajasthan (http://aiccip.cicr.org.in/Sriganganagar.html), 11: Haryana Agricultural University (HAU), Hisar, Haryana (http://aiccip.cicr.org.in/Hisar.html), 12: Central Tobacco Research Institute (CTRI), Rajamundry Andhra Pradesh (http://www.ctri.org.in/), 13: University of agriculture and science (UAS),GKVK, Bangalore, Karnataka (http://www.uasbangalore.edu.in/asp/projects.asp), 14: Jamia Millia Islamia University (JMIU), New Delhi (http://www.jmi.ac.in/), 15: Indian agricultural research institute (IARI), New Delhi (http://www.iari.res.in/index .php?option=com_contentandview=articleandid=179andItemid=525), 16: Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu (http://www.tnau.ac.in/), 17: Babasaheb Bhimrao Ambedkar University (BBAU), Lucknow, U.P. (www.bbau.ac.in/), 18: Jawahar Lal University (JNU), New Delhi (www.jnu.ac.in/), 19: Indian Institute of Technology (IIT), New Delhi (www.iitd.ac.in/), 20: Madurai Kamraj University (MKU),Tamil Nadu (www.mkuniversity.org/), 21: Indian Institute of Horticulture Research (IIHR), Bangalore, Karnataka (www.iihr.ernet.in/), 22: Central Institute of Medicinal and Aromatic Plants (CIMAP), Hyderabad Andhra Pradesh 9 www.cimap.res.in/), 23: University of Kalyani, Kalyani, West Bengal (www.klyuniv.ac.in/), 24: Punjab Agricultural University, Ludhiana, Punjab (www.pau.edn/), 25: Central Arid Zone Research Institute (CAZRI), Jodhpur, Rajasthan (www.cazri.res.in/), 26: Aligarh Mnslim University, Aligarh, U.P. (www.amu.ac.in/), 27: Anand Agricultural University, Anand, Gujarat (www.aau.in/), 27: Central Potato Research Institute (CPRI), Shimla, H.P. (cpri.ernet.in/), 28: IARI Regional Station, Kalimpong, West Bengal (http://www.iari.res.in/index.php? option=com_contentandview=articleandid=461andItemid=1265), 29: Sugarcane Research Station Kunraghat, Gorakhpur, U.P

Two research projects are going at Mody Institute of Technology and Science (MITS), Lakshmangarh, Rajasthan entitled 'RNAi mediated resistance against *Begomovirus*' (DBT funded Project No. BT/PR13129/ GBD/27/197/2009) and "Coordinate approach to creating Transgenic Cotton resistant against Begomoviruses" (DST project no. SR/FT/LS-o42/2009). Around 53 various *Begomovirus* sequences were submitted in NCBI. Their various Genebank Accession Numbers are as follows: HQ631429-HQ631431, JN000700-JN000703, NC-015631, JF968443, JF968444, JN009664-JN009667, HQ876467, JN998441-JN998453, JQ407224, JQ693136-JQ693151. The submitted sequences are of *Begomovirus* infecting ornamental plants such as *Alternanthera sessilis*, *Clerodendrum inerme*, *Calendula officinalis* etc. (Fig. 7) and also include some weeds and crops plants such as *Sonchus asper*, *Datura inoxia*, *Capsicum* sp. etc. (Fig. 8).

Research on *Geminivirus* Recombination (Marwal *et al.*, 2012a), Homology modeling and docking studies are also being carried out on ornamental plants, weeds and crops (Prajapat *et al.*, 2011). A number of first disease reports have also being discovered such as *Begomovirus* infecting *Datura inoxia*, *Mimosa pudica* (Gaur *et al.*, 2011; Marwal *et al.*, 2012b).

Fig. 7(a-b): *Begomovirus* symptoms of (a) Leaf curl disease in *Alternanthera sessilis* and (b) Mosaic symptoms on *Clerodendrum inerme*

Fig. 8(a-b): *Begomovirus* symptoms of (a) Vein yellowing and mosaic on *Sonchus asper* and (b) Leaf curl disease in *Capsicum* species

Geminivirus Database (GVDB) has also being developed at MITS. The objective of the *Geminivirus* Database (GVDB) was to design and provide tool for genetic and *In silico* analysis of *Geminivirus* and its genera *Begomovirus*. It provides a platform for the current status and research on *Geminivirus* especially with reference to *Begomovirus* infection in different plants across India. The GVDB comprises of partial and complete nucleotide sequences of weed, ornamentals etc infecting begomoviruses. Homology modeling and docking results of different begomoviral proteins (cds) were also enclosed. Homology modeling is a bright field to understand and study the genetic diversity of begomoviruses. It involves the prediction of various proteins and helpful to biologist to develop resistance against various Geminiviruses (Prajapat *et al.,* 2010).

This database is endowed with comprehensive information about *Geminivirus* members that grounds infection in various plants species in India assorting from ornamentals plants to common weeds and other plants. The home page of this database offers various links associated with current research programmes, future proposed direction of *Geminivirus* research. The programming and platform of GVDB was developed by perfect Info desk Pvt. Ltd., Jaipur, Rajasthan (India). Domain name www.wikiGeminivirus.org (domain id: D161538660-LROR) was registered at server ns195.ehostpros.com and developed by using online applications of Joomla because it is an open source Content of Management System (CMS). Results of research were used for the designing on MySQL 4.1 (www.mysql.com) by using PHP 5.2 (Hypertext Preprocessor, www.php.net) as front end and the computing platform on Linux.

DIVERSITY AMONG INDIAN *Geminivirus*

India has a vast diversity of Geminiviruses. Some infections are from Indian origin and some comes across the border from the adjacent countries such as Pakistan, Sri Lanka, Bangladesh and other adjacent countries. Table 1 shows almost all the reported Geminiviruses across India (Fauquet *et al.*, 2008).

Table 1: List of reported Geminiviruses in India

Species/virus name	Accession number	Abbreviation
Begomovirus		
Bhendi yellow vein mosaic virus (Okra yellow vein mosaic virus)		
Bhendi yellow vein mosaic virus - India [India:Madurai]	AF241479	BYVMV-IN[IN:Mad]
Chilli leaf curl virus		
Chilli leaf curl virus-A [India::05]	DQ673859	ChiLCV-A[IN::05]
Chilli leaf curl virus-India [India:Papaya:2005]	DQ989326	ChiLCV-IN[IN:Pap:05]
Chilli leaf curl virus-India [India:Varanasi:2006]	EF190217	ChiLCV-IN[IN:Var:06]
Chilli leaf curl virus-India India:PRM:Tomato :2005]	DQ629103	ChiLCV-IN[IN:PRM:Tom:05]
Cotton leaf curl Bangalore virus		
Cotton leaf curl Bangalore virus-[India:Bangalore:2004]	AY705380	CLCuBV-[IN:Ban:04]
Cotton leaf curl Kokhran virus		
Cotton leaf curl Kokhran virus-Manisal [India:Dabawali]	AY456683	CLCuKV-Man[IN:Dab]
Cotton leaf curl Multan virus		
Cotton leaf curl Multan virus-Bhatinda [India:Bhatinda]	DQ191160	CLCuMV-Bha[IN:Bha]
Cotton leaf curl Multan virus-Hisar [India:Hisar:1999]	AY765253	CLCuMV-His[IN:His:99]
Cotton leaf curl Multan virus-Hisar [India:Ludhiana:1999]	AY765257	CLCuMV-His[IN:Lud:99]
Cotton leaf curl Multan virus-Hisar [India:New Delhi:1999]	AY765256	CLCuMV-His[IN:ND:99]
Cotton leaf curl Multan virus-India [India:Abohar:2003]	AY795606	CLCuMV-IN[IN:Abo:03]
Cotton leaf curl Multan virus-India [India:Hisar:2003]	AY795607	CLCuMV-IN[IN:His:03]
Cotton leaf curl Multan virus-India [India:New Delhi2:2003]	AY795605	CLCuMV-IN[IN:ND2:03]

Table 1: Continue

Species/virus name	Accession number	Abbreviation
Cotton leaf curl Multan virus-India [India:Sirsa:1999]	AY765254	CLCuMV-IN[IN:Sir:99]
Cotton leaf curl Multan virus-India [India:Sriganganagar:1994]	AF363011	CLCuMV-IN[IN:Sri:94]
Croton yellow vein mosaic virus		
Croton yellow vein mosaic virus-[India]	AJ507777	CYVMV-[IN]
Dolichos yellow mosaic virus		
Dolichos yellow mosaic virus-[India:Bangalore:2004]	AM157412	DoYMV-[IN:Ban:04]
Dolichos yellow mosaic virus-[India:Bangalore2:2004]	AM157413	DoYMV-[IN:Ban2:04]
Dolichos yellow mosaic virus-[India:Mysore]	AJ875159	DoYMV-[IN:Mys]
Dolichos yellow mosaic virus-[India:Mysore;2004]	AJ968370	DoYMV-[IN:Mys;04]
Dolichos yellow mosaic virus-[India:New Delhi:2000]	AY309241	DoYMV-[IN:ND:00]
Horsegram yellow mosaic virus		
Horsegram yellow mosaic virus-[India:Coimbatore]	AJ627904	HgYMV-[IN:Coi]
	AJ627905	
Indian cassava mosaic virus		
Indian cassava mosaic virus-India [India:Maharashstra 2:1988]	AY730035 AY730036	ICMV-IN[IN:Mah2:88]
Indian cassava mosaic virus-India [India:Maharashstra:1988]	AJ314739	ICMV-IN[IN:Mah:88]
	AJ314740	
Indian cassava mosaic virus-India [India:Trivandrum:1986]	Z24758	ICMV-IN[IN:Tri:86]
	Z24759	
Indian cassava mosaic virus-Kerala [India:Kerala 2:2002]	AJ575819	ICMV-Ker[IN:Ker2:02]
Indian cassava mosaic virus-Kerala [India:Kerala 3:2002]	AJ575820	ICMV-Ker[IN:Ker3:02]
Indian cassava mosaic virus-Kerala [India:Kerala 6:2002]	AJ512823	ICMV-Ker[IN:Ker6:02]
Mungbean yellow mosaic India virus		
Mungbean yellow mosaic India virus-[India:Akola]	AY271893 AY271894	MYMIV-[IN:Ako]
Mungbean yellow mosaic virus-[India:Anand:Cowpea MBKA25:2005]	AY937195 AY937196	MYMIV-[I:Ana:CpMBKA2:05]
Mungbean yellow mosaic India virus-[India:New Delhi:Blackgram 3:1991]	AF126406 AF142440	MYMIV-[IN:ND:Bg3:91]
Mungbean yellow mosaic India virus-[India:Punjab:2005]	DQ400847	MYMIV-[IN:Pun:05]
Mungbean yellow mosaic India virus-[India:New Delhi:Cowpea:2004]	AY939925	MYMIV-[IN:ND:Cp:04]
Mungbean yellow mosaic India virus-[India:New Delhi:Cowpea:2005]	DQ389153	MYMIV-[IN:ND:Cp:05]
Mungbean yellow mosaic India virus-[India:New Delhi:Cowpea 7:1998]	AF481865 AF503580	MYMIV-[IN:ND:Cp7:98]
Mungbean yellow mosaic India virus-[India:Jabalpur]	AJ416349	MYMIV-[IN:Jab]
	AJ420331	
Mungbean yellow mosaic India virus-[India:Kanpour:Cowpea:2005]	DQ389154	MYMIV-[IN:Kan:Cp:05]
Mungbean yellow mosaic India virus-[India:New Delhi:Soybean 2:1999]	AY049772 AY049771	MYMIV-[IN:ND:Sb2:99]
Mungbean yellow mosaic India virus-[India:Varanasi:Cowpea]	AY618902	MYMIV-[IN:Var:Cp]
Mungbean yellow mosaic India virus-[India:Varanasi:Dolichos]	AY547317 DQ061273	MYMIV-[IN:Var:Dol]
Mungbean yellow mosaic India virus-[India: Sriganganagar:Mungbean 1:1996]	AF416742 AF416741	MYMIV-[IN:Sri:Mg1:96]
Mungbean yellow mosaic virus		
Mungbean yellow mosaic virus-[India:Haryana:2001]	AY271896	MYMV-[IN:Har:01]
Mungbean yellow mosaic virus-[India:Madurai:Soybean 2]	AJ582267	MYMV-[IN:Mad:Sb2]
Mungbean yellow mosaic virus-[India:Madurai:Soybean]	AJ421642	MYMV-[IN:Mad:Sb]
	AJ867554	
Mungbean yellow mosaic virus-[India:Maharashstra:Soybean:1999]	AF314530	MYMV-[IN:Mah:Sb:99]
Mungbean yellow mosaic virus-[India:Namakkal B1:2005]	DQ865202	MYMV-[IN:NamB1:05]
Mungbean yellow mosaic virus-[India:Namakkal B2:2005]	DQ865203	MYMV-[IN:NamB2:05]
Mungbean yellow mosaic virus-[India:Vamban:2005]	DQ400848 DQ400849	MYMV-[IN:Vam:05]
Mungbean yellow mosaic virus-[India:Vamban:Vigna KA21]	AJ439059	MYMV-[IN:Vam:VigKA21]
Mungbean yellow mosaic virus-[India:Vamban:Vigna KA27]	AF262064	MYMV-[IN:Vam:VigKA27]

Table 1: Continue

Species/virus name	Accession number	Abbreviation
Mungbean yellow mosaic virus-[India:Vamban:Vigna KA28]	AJ439058	MYMV-[IN:Vam:VigKA28]
Mungbean yellow mosaic virus-[India:Vamban:Vigna KA34]	AJ439057	MYMV-[IN:Vam:VigKA34]
Mungbean yellow mosaic virus-[India:Vigna]	AJ132575	MYMV-[IN:Vig]
	AJ132574	
Papaya leaf curl virus		
Papaya leaf curl virus-India [India:Lucknow]	Y15934	PaLCuV-IN[IN:Luc]
Radish leaf curl virus		
Radish leaf curl virus-[India:Varanasi:2005]	EF175733	RaLCV-[IN:Var:03]
Sida yellow vein Madurai virus		
Sida yellow vein Madurai virus-[India:Madurai:2005]	AM259382	SiYVMaV-[IN:Mad:05]
Squash leaf curl China virus		
Squash leaf curl China virus-India [India: Coimbatore:Pumpkin]	AY184487 AY184488	SLCCNV-IN[IN:Coi:Pum]
Squash leaf curl China virus-India [India:Lucknow:Pumpkin]	DQ026296	SLCCNV-IN[IN:Luc:Pum]
Sri Lankan cassava mosaic virus		
Sri Lankan cassava mosaic virus-India [India:Adivaram]	AJ579307	SLCMV-IN[IN:Adi]
	AJ579308	
Sri Lankan cassava mosaic virus-India [India:MuvattupuCha:2004]	AJ575820	SLCMV-IN[IN:Muv:04]
Sri Lankan cassava mosaic virus-India [India:Kattukuda]	AJ575821	SLCMV-IN[IN:Kat]
Sri Lankan cassava mosaic virus-India [India:Kerala 15]	AJ890224	SLCMV-IN[IN:Ker15]
Sri Lankan cassava mosaic virus-India [India:Kerala 17]	AJ890225	SLCMV-IN[IN:Ker17]
Sri Lankan cassava mosaic virus-India [India:Kerala C4]	AJ890226	SLCMV-IN[IN:KerC4]
Sri Lankan cassava mosaic virus-India [India:Salem]	AJ607394	SLCMV-IN[IN:Sal]
Sri Lankan cassava mosaic virus-India [India:Tamil Nadu 2]	AJ890227	SLCMV-IN[IN:Tam2]
Sri Lankan cassava mosaic virus-India [India:Tamil Nadu 6]	AJ890228	SLCMV-IN[IN:Tam6]
Sri Lankan cassava mosaic virus-India [India:Tamil Nadu 7]	AJ890229	SLCMV-IN[IN:Tam7]
Tomato leaf curl Bangalore virus		
Tomato leaf curl Bangalore virus-A [India:Bangalore 1]	Z48182	ToLCBV-A[IN:Ban1]
Tomato leaf curl Bangalore virus-A [India:Kerala IV:2005]	DQ887537	ToLCBV-A[IN:KerIV:05]
Tomato leaf curl Bangalore virus-A [India:Kolar]	AF428255	ToLCBV-A[IN:Kol]
Tomato leaf curl Bangalore virus-B [India:Bangalore 5]	AF295401	ToLCBV-B[IN:Ban5]
Tomato leaf curl Bangalore virus-B [India:Fatehabad:Cotton]	AY456684	ToLCBV-B[IN:Fat:Cot]
Tomato leaf curl Bangalore virus-C [India:Bangalore 4:1997]	AF165098	ToLCBV-C[IN:Ban4:97]
Tomato leaf curl Bangalore virus-C [India:Bangalore:AVT1]	AY428770	ToLCBV-C[IN:Ban:AVT1]
Tomato leaf curl Gujarat virus		
Tomato leaf curl Gujarat virus-[India:Mirzapur:1999]	AF449999	ToLCGV-[IN:Mir:99]
Tomato leaf curl Gujarat virus-[India:Vadodara:1999]	AF413671	ToLCGV-[IN:Vad:99]
Tomato leaf curl Gujarat virus-[India:Varanasi:2001]	AY190290 AY190291	ToLCGV-[IN:Var:01]
Tomato leaf curl Joydebpur virus		
Tomato leaf curl Joydebpur virus-India [India;Kalyani;2006]	EF194765	ToLCJoV-IN[IN;Kal;06]
Tomato leaf curl Kerala virus		
Tomato leaf curl Kerala virus-[India:Kerala II:2005]	DQ852623	ToLCKeV-[IN:KerII:05]
Tomato leaf curl Karnataka virus		
Tomato leaf curl Karnataka virus-Bangalore [India:Bangalore:1993]	U38239	ToLCKV-Ban[IN:Ban:93]
Tomato leaf curl Karnataka virus-Janti [India:Janti:2005]	AY754812	ToLCKV-Jan[IN:Jan:05]
Tomato leaf curl New Delhi virus		
Tomato leaf curl New Delhi virus-India[India:Meerut:Potato:2005]	EF043231	ToLCNDV-IN[IN:Mer:Pot:05]
	EF043232	

Table 1: Continue

Species/virus name	Accession number	Abbreviation
Tomato leaf curl New Delhi virus-India[India:Happur:Potato:2005]	EF043230 EF043233	ToLCNDV-IN[IN:Hap:Pot:05]
Tomato leaf curl New Delhi virus-India [India:Hissar:Cotton:2005]	EF063145	ToLCNDV-IN[IN:His:Cot:05]
Tomato leaf curl New Delhi virus-India [India:Lucknow]	Y16421 X89653	ToLCNDV-IN[IN:Luc]
Tomato leaf curl New Delhi virus-India [India:Meerut:Potato	AY286316 AY158080	ToLCNDVIN[IN:Mee:Po12:02]
	12:2002]	
Tomato leaf curl New Delhi virus-India [India:New Delhi:2005]	DQ169056 DQ169057	ToLCNDV-IN[IN:ND:05]
Tomato leaf curl New Delhi virus-India [India:New Delhi:AVT1]	AY428769 AY438563	ToLCNDV-IN[IN:ND:AVT1]
Tomato leaf curl New Delhi virus-India [India:New Delhi:Mild:1992]	U15016	ToLCNDV-IN[IN:ND:Mld:92]
Tomato leaf curl New Delhi virus-India [India:New Delhi:Severe:1992]	U15015 U15017	ToLCNDV-IN[IN:ND:Svr:92]
Tomato leaf curl New Delhi virus-India [India:Sonepat:Luffa:2005]	AY939926 AY939924	ToLCNDV-IN[IN:Son:Luf:05]
Tomato leaf curl New Delhi virus-Papaya [India:New Delhi: Papaya:2005]	DQ989325	ToLCNDV-Pap[IN:ND:Pap:05]
Tomato leaf curl Pune virus		
Tomato leaf curl Pune virus-[India:Pune:2005]	AY754814	ToLCBV-[IN:Pun:05]
Tomato leaf curl Rajasthan virus		
Tomato leaf curl Rajasthan virus-[India:Rajasthan:2005]	DQ339117	ToLCBV-[IN:Raj:05]
Veruonia yellow vein virus		
Veruonia yellow vein virus-[India:Madurai:2005]	AM182232	VeYVV-[IN:Mad:05]
Unassigned isolates in the genus		
Guar mosaic virus		GMV
Jatropha mosaic virus		JMV
Okra leaf curl virus-India		OkLCuIV
Tobacco leaf curl virus-India	AB001292-8 AB001301-4 AB001307-20	TbLCIV
Tomato leaf curl India virus	L11746	ToLCIV
Bhendi yellow vein Bhubhaneswar virus	NC_012041	
Bhendi yellow vein Delhi virus	NC_011919	
Tomato leaf curl Palampur virus	FJ660442	
Tomato leaf curl Kerman virus		
Tomato leaf curl Patna virus	NC_012492	
Tobacco leaf curl Lucknow virus	GU253915	
Unnamed Indian mentha *Begomovirus*		
Mastrevirus		
Unassigned isolates in the genus		
Bajra streak virus		BaSV

Geminiviruses causes a variety of symptoms and exhibits high rate of recombination and pseudo-recombination that contributes in the evolution of new virus strains/species (Pita *et al.*, 2001). Inter-specific recombination has resulted in remarkable diversity among Geminiviruses (Garrido-Ramirez *et al.*, 2000) and that is the major cause of the emergence of new *Geminivirus* diseases in tropical and subtropical regions. In some cases, the recombinants exhibited a new pathogenic phenotype which is often more virulent than the parents, many research reports have demonstrated evidence of recombination in a wide range of Geminiviridae genera (Marwal *et al.*, 2012a).

RNAi TECHNOLOGY: RAY OF HOPE

RNAi-mediated virus resistance was first reported against Potato virus Y (PVY) in transgenic tobacco plant (Waterhouse *et al.*, 1998). RNAi technology was used as an antiviral approach against human cell lines (Novina *et al.*, 2002) but it can be used for developing resistance against plant viruses (Waterhouse *et al.,* 2001). RNAi technology when used against a *Geminivirus* (African cassava mosaic virus (ACMV)) showed 99% decrease of Rep transcripts and 66% reduction in viral DNA. Here in it the siRNA were transiently transferred into the protoplast making it effective against the replicase (Rep)-coding sequence of the ACMV (Vanitharani *et al.*, 2003).

In view of the fact that the unearthing of RNAi in *Caenorhabditis elegans* which provide as an experiment archetypical and its study in *Drosophila melanogaster* and other organisms, scores of mechanism for RNA interference has been recommended (Montgomery *et al.*, 1998). RNA interference is a phenomenon primarily for the regulation of gene expression; self or non self depending upon the surrounding factors or conditions, with the help of RNA molecules that are non coding in nature to control cellular metabolism or simply called post transcriptional gene silencing (Dhakar *et al.*, 2010; Chang *et al.,* 2012). Short interfering RNAs (siRNAs), the 21-to 23-nt double-stranded intermediates of this natural defense mechanism, are becoming powerful tools for reducing gene expression and countering viral infection in a variety of cells (Rougemaille *et al.*, 2012).

The DICER complex which is RNAase III like enzymes (Bernstein *et al.*, 2001) responsible for RNAi pathway exists in two forms: Dcr-1/Loquacious (Loqs)-PB (also known as R3D1-L[long]): generate miRNA and Dcr-2/R2D2 generates siRNA. Dcr-1 is an enzyme that shows ATP independent functions (Lee *et al.*, 2004) and affinity towards stem-loop form of RNA (precursor of miRNA). Dcr-2 shows ATP dependent activity with substrate specificity to double stranded RNA. R2D2 contains 2 ds RNA binding domains (ds RBDs). Dcr2/r2d2 heterodimer binds to siRNA forms RDI complex (R2D2 Dcr Initiator complex) and helps it to load into RNA silencing effector complex such as RNA-induced silencing complex called as RISC (Hammond *et al.*, 2000) by forming (RLC) RISC Loading Complex. RLC then recruits and associates with Ago 2 protein forming holo-RISC from Pre-RISC after the removal of passenger strand (Van Mierlo *et al.,* 2012).

The holo-RISC then acts as the effective machinery for gene silencing. As the guide strand of RISC binds with the corresponding mRNA, the endonuclease activity of the complex degrades the mRNA thereby resulting in Post Transcriptional Gene Silencing (PTGS) (Liu *et al.*, 2012). RNA interference silencing mechanism could amplify its signal intensity, termed as Transitivity. A role of RNA dependent RNA polymerase (RdRP) was assumed in this mechanism (Zhang *et al.*, 2012). Two models were proposed: (1) RdRP generates the newly synthesized siRNA directly (2) RdRP use siRNA as a template for the production of long dsRNA, which are then cleaved into siRNA. Mobility of the silencing signal termed as system is spread by specific proteins moving across plasmodesmata in phloem thus activating RNAi (Himber *et al.*, 2003).

Targeting Rep and AV_2 gene by antisense technology is found to be quite successful (Sanjaya *et al.*, 2005). To make PTGS an effective method both sense and antisense RNAs is a prerequisite and a transgenic tobacco (*Nicotiana benthamiana*) using RNAi was developed (Dasgupta *et al.*, 2003). Using RNA silencing approach AC2 protein of mungbean yellow mosaic India virus (MYMIV) was effectively suppressed (Singh *et al.*, 2007). Reduction in rep mRNA of MYMIV was achieved by the catalytic activity of both active and inactive ribozyme which is primarily due to the of Rep-siRNAs formation in presence of the ribozymes (Chilakamarthi *et al.*, 2007).

The hairpin construct of coat protein made, has 35 S promoter (1.3 kb), sense sequence of MYMIV (130 bp) followed by intron (741 bp), after which the antisense (130 bp) target sequence and OCS terminator (765 bp) were cloned in cloning vector pHANNIBAL. Results indicated that, co-agro inoculation of coat protein hairpin construct (Cphp) prevent viral pathogenesis (Kumari and Malathi, 2012). Success has also achieved in developing resistance against AC1 and AC4 viral genes of tomato leaf curl virus. Moreover transgenic lines of popular potato cultivars Kufri Badshah and Kufri Pukhraj with increased potato apical leaf curl virus (PALCV) resistance using RNAi technology targeting the replication associated protein gene (AC1) of the virus were also developed (Tomar *et al.,* 2012). Strategies like Intron-spliced hairpin RNAs, small hairpin RNAs, self-complementary inverted repeats and antisense technology were used to developed resistance (Ramesh *et al.,* 2007). Transgenic resistance against begomoviruses has been achieved in a number of plants using a variety of strategies. The results presented above showed that the RNAi approach has been investigated extensive which is a powerful tool for biochemical studies for developing transgenic plants and a ray of hope for various challenging geminiviral diseases.

CONCLUSION AND FUTURE PROSPECTS

India is an agriculture based country. A wide variety of plants species are grown in India such as economically important crops and ornamentals plants. Indian weather is tropical in nature and is very much suitable for the prevalence of white fly which is the main vector for causing *geminiviral* infections. Due to which a number of new viral species have being found in India, includes a wide range of information mentioning the new disease reports from throughout India. Indian *Begomovirus* have a diverse kind of host range for example tomato leaf curl virus was found infecting *Crotalaria juncea*, *Solanum tuberosum* and *Cyamopsis tetragonoloba*. Similarly radish leaf curl virus was found infecting okra.

There are also reports which enlightens the prevalence of *Begomovirus* from neighboring countries such as Pakistan, Sri Lanka, Bangladesh etc for example tomato leaf curl Bangladesh virus was found infecting *Gaillardia*, tomato leaf curl Pakistan virus was found infecting *Mentha spicata*. An expected consequence of this scenario would be recombination which plays an important role for the evolution of new *Geminivirus* strains in India and these new strains are responsible for heavy loss of new host variety. Thus there is an urgent need of controlling *Begomovirus* infections. The use of computational and molecular techniques e.g., RNAi is a potential tools for reducing the prevalence of various *Geminivirus* diseases. Results of these techniques effectively applied for disease management, crop protection and development of quarantine strategies at state and national level in India.

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REFERENCES

AVRDC, 2012. The great pyramid: By stacking multiple genes for resistance, AVRDC plant breeders develop tomatoes that are a monument to better pest control. The World Vegetable Center. http://avrdc.org/?p=51

Alberter, B., M. Ali Rezaian and H. Jeske, 2005. Replicative intermediates of tomato leaf curl virus and its satellite DNAs. Virology, 331: 441-448.

Baliji, S., M.C. Black, R. French, D.C. Stenger and G. Sunter, 2004. Spinach curly top virus: A newly described *Curtovirus* species from southwest texas with incongruent gene phylogenies. Phytopathol., 94: 772-779.

Bernstein, E., A.A. Caudy, S.M. Hammond and G.J. Hannon, 2001. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature, 409: 363-366.

Bhattacharyya, P. and G. Chakraborty, 2005. Current status of organic farming in India and other countries. Indian J. Fertilizers, 1: 111-123.

Bisaro, D., 1996. Geminivirus DNA Replication. In: DNA Replication in Eukaryotic Cells, Depamphilis, M.L. (Ed.). Cold Spring Harbor Laboratory Press, New York USA., pp: 833-854.

Bolok Yazdi, H.R., J. Heydarnejad and H. Massumi, 2008. Genome characterization and genetic diversity of beet curly top Iran virus: A *Geminivirus* with a novel nonanucleotide. Virus Genes., 36: 539-545.

Boulton, M.I., 2002. Functions and interactions of mastrevirus gene products. Physiol. Mol. Plant Pathol., 60: 243-255.

Briddon, R.W. and J. Stanley, 2006. Sub-viral agents associated with plant single stranded DNA viruses. Virology, 344: 198-210.

Briddon, R.W. and P.G. Markham, 2001. Complementation of bipartite *Begomovirus* movement functions by topocuviruses and *Curtoviruses*. Arch. Virology, 146: 1811-1819.

Briddon, R.W., S. Mansoor, I.D. Bedford, M.S. Pinner and K. Saunders *et al*., 2001. Identification of DNA components required for induction of cotton leaf curl disease. Virology, 285: 234-243.

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

Chang, S.S., Z. Zhang and Y. Liu, 2012. RNA interference pathways in fungi: Mechanisms and Functions. Ann. Rev. Microbiol., (In press).

Chasan, R., 1995. *Geminivirus*es: A twin approach to replication. Plant Cell, 7: 659-661.

Chilakamarthi, U., S.K. Mukherjee and J.K. Deb, 2007. Intervention of geminiviral replication in yeast by ribozyme mediated downregulation of its rep protein. FEBS Lett., 581: 2675-2683.

CRSP, 2012. Feeding more people with healthier crops. Proceedings of the Virology Symposium, June 12, 2012, IPM CRSP, India.

Dasgupta, I., V.G. Malathi and S.K. Mukherjee, 2003. Genetic engineering for virus resistance. Curr Sci., 84: 341-354.

Dhakar, K., V.K. Gupta, M.S. Rathore and R.K. Gaur, 2010. Virus resistance and gene silencing in plants infected with *Begomovirus*. J. Applied Sci., 10: 1787-1791.

Fauquet, C.M., M.A. Mayo, J. Maniloff, U. Desselberger and L.A. Ball, 2005. Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, USA., pp: 1259.

Fauquet, C.M., R.W. Briddon, J.K. Brown, E. Moriones, J. Stanley, M. Zerbini and X. Zhou, 2008. *Geminivirus* strain demarcation and nomenclature. Arch. Virol., 153: 783-821.

Garrido-Ramirez, E.R., M.R. Sudarshana and R.L. Gilbertson, 2000. Bean golden yellow mosaic virus from Chiapas, Mexico: Characterization, pseudorecombination with other bean-infecting *Geminivirus*es and germ plasm screening. Phytopathology, 90: 1224-1232.

Gaur, R.K., R. Prajapat, A. Marwal, A. Sahu and M.S. Rathore, 2011. First report of a *Begomovirus* infecting *Mimosa pudica* in India. J. Plant Pathol., Vol. 93, No. 4.

Ghosh, R., S. Paul, S. Das, P. Palit and S. Acharyya *et al.*, 2008. Molecular evidence for existence of a new world *Begomovirus* associated with yellow mosaic disease of *Corchorus capsularis* in India. Australasian Plant Dis. Notes, 3: 59-62.

Govindappa, M.R., I. Shankergoud, K.S. Shankarappa, W.A.R.T. Wickramaarachchi, B.A. Reddy and K.T. Rangaswamy, 2011. Molecular detection and partial characterization of *Begomovirus* associated with leaf curl disease of sunflower (*Helianthus annuus*) in Southern India. Plant Pathol. J., 10: 29-35.

Gutierrez, C., 2000. DNA replication and cell cycle in plants: Learning from Geminiviruses. EMBO J., 19: 792-799.

Gutierrez, C., E. Ramirez-Parra, M.M. Castellano, A.P. Sanz-Burgos, A. Luque and R. Missich, 2004. *Geminivirus* DNA replication and cell cycle interactions. Vet. Microbiol., 98: 111-119.

Hammond, S.M., E. Bernstein, D. Beach and G.J. Hannon, 2000. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature, 404: 293-296.

Hanley-Bowdoin, L., S.B. Settlage, B.M. Orozco, S. Nagar and D. Robertson, 1999. Geminiviruses: Models for plant DNA replication, transcription and cell cycle regulation. Crit. Rev. Plant Sci., 18: 71-106.

Heyraud-Nitschke, F., S. Schumacher, J. Laufs, S. Schaefer, J. Schell and B. Gronenborn, 1995. Determination of the origin cleavage and joining domain of *Geminivirus* Rep proteins. Nucleic Acids Res., 23: 910-916.

Himber, C., P. Dunoyer, G. Moissiard, C. Ritzenthaler and O. Voinnet, 2003. Transitivity-dependent and -independent cell-to-cell movement of RNA silencing. EMBO J., 22: 4523-4533.

Hunter, W.B., E. Hierbert, S.E. Webb, J.H. Tsai and J.E. Polston, 1998. Location of Geminiviruses in the whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae). Plant Dis., 82: 1147-1151.

John, P., P.N. Sivalingam, N. Kumar, A. Mishra, Y.S. Ahlawat and V.G. Malathi, 2006. A new *Begomovirus* associated with yellow mosaic disease of *Clerodendron inerme*. Plant Pathol., 55: 291-291.

Kaliappan, K., N.R. Choudhury, G. Suyal and S.K. Mukherjee, 2012. A novel role for RAD54: This host protein modulates geminiviral DNA replication. FASEB J., 26: 1142-1160.

Kataria, R. and D. Kumar, 2012. Occurrence and infestation level of sucking pests: Aphids on various host plants in agricultural fields of vadodara, Gujarat (India). Int. J. Scientific Res. Publicat., 2: 1-6.

Khan, A.A., Q.A. Naqvi, M.S. Khan, R. Singh and S.K. Raj, 2005. First report of a *Begomovirus* infecting *Calendula* in India. Plant Pathol., 54: 569-569.

Khan, J.A., M.K. Siddiqui and B.P. Singh, 2002. The natural occurrence of a *Begomovirus* in sunn hemp (*Crotalaria juncea*) in India. Plant Pathol., 51: 398-398.

Khan, J.A., S.S. Sohrab and A. Aminuddin, 2003. Guar leaf curl disease from India is caused by tomato leaf curl virus. Plant Pathol., 52: 796-796.

Kumar, J., A. Kumar, S.P. Singh, J.K. Roy and A. Lalit *et al.*, 2012a. First report of Radish leaf curl virus infecting okra in India. New Dis. Rep., (In press).

Kumar, R.V., A.K. Singh and S. Chakraborty, 2012b. A new monopartite *Begomovirus* species, *Chilli leaf curl Vellanad virus* and associated betasatellites infecting chilli in the Vellanad region of Kerala, India. New Dis. Rep., (In press).

Kumari, A. and V.G. Malathi, 2012. RNAi-Mediated strategy to develop transgenic resistance in grain legumes targeting the *Mungbean Yellow Mosaic India Virus* Coat Protein Gene. Proceedings of the International Conference on Plant Biotechnology for Food Security: New Frontiers, February 21-24, 2012, New Delhi, India .

Kurth, E.G., V.V. Peremyslov, A.I. Prokhnevsky, K.D. Kasschau, M. Miller, J.C. Carrington and V.V. Dolja, 2012. Virus-derived gene expression and RNA interference vector for grapevine. J. Virol., 10.1128/JVI.00436-12

Lee, I.M., R.E. Davis and D.E. Gundersen-Rindal, 2000. Phytoplasma: Phytopathogenic mollicutes. Ann. Rev. Microbiol., 54: 221-555.

Lee, Y.S., K. Nakahara, J.W. Pham, K. Kim, Z. He, E.J. Sontheimer and R.W. Carthew, 2004. Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. Cell, 117: 69-81.

Liu, Y., H. Zhai, K. Zhao, B. Wu and X. Wang, 2012. Two suppressors of RNA silencing encoded by cereal-infecting *Luteoviridae*. J. Gen. Virol., 93: 1825-1830.

Mahatma, L. and M.K. Mahatma, 2012. First report of a variant of tomato leaf curl Bangladesh virus infecting *Gaillardia*. New Dis. Rep., vol, 26, 10.5197/j.2044-0588.2012.026.004

Mandal, B., S. Mandal, S.S. Sohrab, K.B. Pun and A. Varma, 2004. A new yellow mosaic disease of chayote in India. Plant Pathol., 53: 797-797.

Markham, P.G., S.L. Bedford and M.S. Pinner, 1994. The transmission of *Geminivirus* by *Bemisia tabaci*. Pesticide Sci., 42: 123-128.

Maruthi, M.N., A.R. Rekha, M.R. Govindappa, J. Colvin and V. Muniyappa, 2006. A distinct *begomovirus* causes Indian dolichos yellow mosaic disease. Plant Pathol., 55: 290-290.

Marwal, A., A. Sahu, R. Prajapat, D.K. Choudhary, R.K. Gaur, 2012a. First report of association of a *Begomovirus* with the leaf curl disease of a common weed, *Datura inoxia*. Indian J. Virol., 23: 83-84.

Marwal, A., R. Prajapat, A. Sahu and R.K. Gaur, 2012b. *In silico* Recombination Analysis: A Study for *Geminivirus* Host Mobility Asian J. Biol. Sci., 5: 1-8.

Medina-Ramos, G., R. De La Torre-Almaraz, R. Bujanos-Muniz, R.G. Guevara-Gonzalez and N. Tierranegra-Garcia *et al*., 2008. Co-transmission of Pepper huastecoyellow vein virus and *Pepper golden* mosaic virus in chili pepper by *Bemisia tabaci* (Genn.). J. Entomol., 5: 176-184.

Meetei, P.A., P. Singh, P. Nongdam, N.P. Prabhu, R.S. Rathore and V. Vindal, 2012. NeMedPlant: A database of therapeutic applications and chemical constituents of medicinal plants from North-East region of India. Bioinformation, 8: 209-211.

Montgomery, M.K., S. Xu and A. Fire, 1998. RNA as a target of double-stranded RNA-mediated genetic interference in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci., 95: 15502-15507.

Nahid, N., I. Amin, S. Mansoor, E.P. Rybicki, E. van der Walt and R.W. Briddon, 2008. Two dicot-infecting mastreviruses (family Geminiviridae) occur in Pakistan. Arch. Virol., 153: 1441-1451.

Novina, C.D., M.F. Murray, D.M. Dykxhoorn, P.J. Beresford and J. Riess *et al*., 2002. siRNA-directed inhibition of HIV-1 infection. Nat. Med., 8: 681-686.

Park, E., H. Hwang and S. Lee, 1999. Molecular analysis of *Geminivirus* ORFs on symptom development. Plant Pathol. J., 15: 38-43.

Park, J., H. Hwang, H. Shim, K. Im, C.K. Auh, S. Lee and K.R. Davis, 2003. Altered cell shapes, hyperplasia and secondary growth in Arabidopsis caused by Beet curly top *Geminivirus* infection. Mol. Cells, 17: 117-124.

Paul, S., R. Ghosh, S. Das, P. Palit and S. Acharyya *et al*., 2009. First report of tomato leaf curl Joydebpur virus and associated betasatellite in kenaf (*Hibiscus cannabinus*) plants showing leaf curl symptoms from Southern India. Plant Pathol., 58: 403-403.

Paul, S., R. Ghosh, A. Roy and S.K. Ghosh, 2012. Analysis of coat protein gene sequences of *begomoviruses* associated with different weed species in India. Phytoparasitica, 40: 95-100.

Pita, J.S., V.N. Fondong, A. Sangare, G.W. Otim-Nape, S. Ogwal and C.M. Fauquet, 2001. Recombination, pseudorecombination and synergism of *Geminiviruses* are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. J. Gen. Virol., 82: 655-665.

Prajapat, R., R.K. Gaur and A. Marwal, 2011. Homology modeling and docking studies between AC1 rep protein of *begomovirus* and whey a-lactalbumin. Asian J. Biol. Sci., 4: 352-361.

Prajapat, R., R.K. Gaur, R. Raizada and V.K. Gupta, 2010. *In silico* analysis of genetic diversity of *begomovirus* using homology modelling. J. Biol. Sci., 10: 217-223.

Raj, S.K., M.S. Khan and R. Singh, 2005. Natural occurrence of a *Begomovirus* on Pigeon pea in India. Plant Pathol., 54: 809-809.

Raj, S.K., M.S. Khan, S.K. Snehi, S. Srivastava and H.B. Singh, 2006. First report of Tomato leaf curl Karnatka virus infecting soybean in India. Plant Pathol., 55: 817-817.

Raj, S.K., S.K. Snehi, M.S. Khan, P. Chandra and R.M. Pandey, 2008. First molecular identification of a *begomovirus* associated with yellow vein net disease in grain amaranth (*Amaranthus cruentus* L.) in India. Aust. Plant Dis. Notes, 3: 129-131.

Ramesh, S.V., A.K. Mishra and S. Praveen, 2007. Hairpin RNA-mediated strategies for silencing of tomato leaf curl virus AC1 and AC4 genes for effective resistance in plants. Oligonucleotides, 17: 251-257.

Reddy, R.V.W., V. Muniyappa, J. Colvin and S. Seal, 2005. A new *Begomovirus* isolated from *Gossypium barbadense* in Southern India. Plant Pathol., 54: 570-570.

Rojas, M.R., C. Hagen, W.J. Lucas and R.L. Gilbertson, 2005. Exploiting chinks in the plants armor: Evolution and emergence of Geminiviruses. Annu. Rev. Phytopathol., 43: 361-394.

Rougemaille, M., S. Braun, S. Coyle, P.A. Dumesic and J.F. Garcia *et al*., 2012. Ers1 links HP1 to RNAi. PNAS, 109: 11258-11263.

Rouhibakhsh, A. and V.G. Malathi, 2005. Severe leaf curl disease of cowpea, a new disease of cowpea in northern India caused by *Mungbean yellow mosaic India virus* and a satellite DNA β. Plant Pathol., 54: 259-259.

Samad, M.K., A.K. Gupta, P.V. Shasany, Ajayakumar and M. Alam, 2009. *begomovirus* related to Tomato leaf curl Pakistan virus newly reported in Mentha crops in India. Plant Pathol., 58: 404-404.

Sanjaya, V.V.S., V. Prasad, N. Kirthi, S.P. Maiya, H.S. Savithri and G.L. Sita, 2005. Development of cotton transgenics with antisense AV2 gene for resistance against Cotton Leaf Curl Virus (CLCuD) via Agrobacterium tumefaciens. Plant Cell Tissue Organ Cult., 81: 55-63.

Saunders, K., A. Lucy and J. Stanley, 1991. DNA forms of the *Geminivirus* African cassava mosaic virus consistent with a rolling circle mechanism of replication. Nucleic Acids Res., 19: 2325-2330.

Saunders, K., R.W. Briddon and J. Stanley, 2008. Replication promiscuity of DNA-β satellites associated with monopartite *Begomoviruses*: Deletion mutagenesis of the Ageratum yellow vein virus DNA-β satellite localizes sequences involved in replication. J. Gener. Virol., 89: 3165-3172.

Senanayake, D.M.J.B., B. Mandal, S. Lodha and A. Varma, 2007. First report of chilli leaf curl virus affecting chilli in India. Plant Pathol., 56: 343-343.

Shih, S.L., W.S. Tsai, S.K. Green and D. Singh, 2007. First report of tomato leaf curl Joydebpur virus infecting chilli in India. Plant Pathol., 56: 341-341.

Sidhu, J.S., R.S. Mann and N.S. Butter, 2009. Deleterious effects of cotton leaf curl virus on longevity and fecundity of whitefly, *Bemisia tabaci* (Gennadius). J. Entomol., 6: 62-66.

Singh, D.K., S. Karjee, P.S. Malik, N. Islam and S.K. Mukherjee, 2007. DNA Replication and Pathogenecity of MYMIV. In: Communicating Current Research and Educational Topics and Trends in Applied Microbiology, Mendez-Vilas, A. (Ed.). FORMATEX, USA., ISBN-13: 9788461194223, pp: 155-162.

Sivalingam, P.N. and A. Varma, 2012. Role of betasatellite in the pathogenesis of a bipartite *begomovirus* affecting tomato in India. Arch. Virol., 157: 1081-1092.

Stanley, J., D.M. Bisaro, R.W. Briddon, J.K. Brown and C.M. Fauquet *et al.*, 2005. Family geminiviridae. In Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses.

Tiendrebeogo, F., V.S.E. Traore, N. Barro, A.S. Traore, G. Konate and O. Traore, 2008. Characterization of Pepper yellow vein mali virus in *Capsicum* sp. in Burkina Faso. Plant Pathol. J., 7: 155-161.

Tomar, G., A. Jeevalatha, G. Thakur, N.N. Sharma and R.K. Shandil *et al.*, 2012. RNAi technology for resistance development against apical leaf curl virus in potato. Proceedings of the International Conference on Plant Biotechnology for Food Security: New Frontiers, February 21-24, 2012, New Delhi, India, pp: 156-156.

Usharani, K.S., B. Surendranath, S.M. Paul-Khurana, I.D. Garg and V.G. Malathi, 2004. Potato leaf curl-a new disease of potato in northern India caused by a strain of tomato leaf curl New Delhi virus. Plant Pathol., 53: 235-235.

Van Mierlo, J.T., A.W. Bronkhorst, G.J. Overheul, S.A. Sadanandan and J.O. Ekstrom *et al.*, 2012. Convergent evolution of argonaute-2 slicer antagonism in two distinct insect RNA viruses. PLoS Pathog., Vol. 8.

Vanitharani, R., P. Chellappan and C. M. Fauquet, 2003. Short interfering RNA-mediated interference of gene expression and viral DNA accumulation in cultured plant cells. Proc. Natl. Acad. Sci. USA, 100: 9632-9636.

Varma, A. and V.G. Malathi, 2003. Emerging *Geminivirus* problems: A serious threat to crop production. Ann. Applied Biol., 142: 145-164.

Venkataravanappa, V., C.N.L Reddy, J. Salil and M.K. Reddy, 2012. Molecular characterization of distinct bipartite *Begomovirus* infecting bhendi (*Abelmoschus esculentus* L.) in India. Virus Genes., 44: 522-535.

Wang, J., X.L. Bing, M. Li, G.Y. Ye and S.S. Liu, 2012. Infection of tobacco plants by a *begomovirus* improves nutritional assimilation by a whitefly. Entomol. Exper. Applicata, 144: 191-201.

Waterhouse, P.M., M.B. Wang and T. Lough, 2001. Gene silencing as an adaptive defense against viruses. Nature, 411: 834-842.

Waterhouse, P.M., M.W. Graham and M.B. Wang, 1998. Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. Proc. Natl. Acad. Sci. USA., 95: 13959-13964.

Xie, Q., A.P. Sanz-Burgos, H. Guo, J.A. Garcia and C. Gutierrez, 1999. GRAB proteins, novel members of the NAC domain family, isolated by their interaction with a *Geminivirus* protein. Plant Mol. Biol., 39: 647-656.

Yadava, P., G. Suyal and S.K. Mukherjee, 2010. *Begomovirus* DNA replication and pathogenicity. Curr. Sci., 98: 360-368.

Zhang, C., T.A. Montgomery, S.E. Fischer, S.M. Garcia and C.G. Riedel *et al.*, 2012. The Caenorhabditis elegans RDE-10/RDE-11 complex regulates RNAi by promoting secondary siRNA amplification. Curr. Biol., 22: 881-890.