GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

Review Article

DEVELOPING RNAi STRATEGY AGAINST YELLOW VEIN MOSAIC DISEASE (YMVD) OF OKRA

*Geetha Shanmugam, Kumar Krish & Balasubramanian Ponnuswami
Department of Plant Biotechnology, Centre for plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641003 (Tamil Nadu), INDIA
*Corresponding author email: geethshan@gmail.com

ABSTRACT
Okra (*Abelmoschus esculentus*; family *Malvaceae*) is grown in temperate as well as subtropical regions of the world, both for human consumption as a vegetable and for industrial uses. Okra yields are affected by the diseases caused by phytopathogenic viruses. India is the largest producer of okra and in this region a major biotic constraint to production are viruses of the genus *Begomovirus*. Yellow vein mosaic disease (YVMD) of bhendi is spreading rapidly throughout India, affecting plants at all growth stages, and resulting in plants yielding unmarketable fruits and clearly a most serious of the newly emerging/reemerging begomovirus-associated diseases in India. Despite its importance, the etiology of YVMD across much of India is unknown and has been shown to be caused by beta satellite associated monopartite begomovirus species. A rich diversity of viruses infecting Indian bhendi is of concern, since this situation undoubtedly increases incidences of mixed infections and increases the possibility of yet more novel recombinant viruses arising within this species. Together with the already existing diversity of begomoviruses infecting bhendi, such novel recombinants will probably further undermine efforts to control the virus using transgenic or inbred resistance strategies. Gene silencing can occur either repression of transcription, termed Transcriptional Gene Silencing (TGS) or through mRNA degradation, termed post transcriptional gene silencing. Transcriptional gene silencing results from a marked decrease in transcription and hypermethylaton of genes affected. RNAi or PTGS discovered as a natural anti-viral system in plants. Studies revealed that geminiviruses which replicates in nucleus can induce PTGS and become the target for it. RNAi has a strong potential to reduce the infection of geminiviruses. Several studies are already conducted on different genes geminiviruses and that are used for generating virus resistance plants. Our main objective of present study is to develop resistance against geminivirus using a novel strategy based on RNAi. Here we summarise how the RNAi mechanism works against begomovirus infection in plants and how we can utilize it to reduce the losses.

KEYWORDS: RNAi, Okra, Yellow vein mosaic disease, *Geminivirus*, *Begomovirus*, Beta satellite.

INTRODUCTION

RNAi also known as RNA silencing and PTGS (Post transcriptional gene silencing), a natural mechanism works against viral infection in both plants and animals (Napoli *et al*., 1990). It is targeted by dsRNA which leads to the degradation of sequence specific viral RNA (Fig. 1) (Hamilton and Baulcombe, 1999). Double strandard RNAs generated by virus are recognized and cleaved into small interfering RNAs of approximately 25 nucleotide by the RNAase III like enzymes (Bass, 2000) called as DICER. There are four dicer like enzymes identified in plants. First involved in miRNA biogenesis (Finnegan *et al*., 2003), second play role in viral siRNA production and third is responsible for transposon siRNA production (Xie *et al*., 2004). These siRNAs incorporate into the RISC (RNA induced silencing complex) and guide the degradation of viral RNA. Many viruses having ssRNA genome which released from the protein coat when they enter a cell. They replicate through virus encoded RNA-dependent RNA polymerase and generate sense and antisense RNA. These RNAs forms dsRNA and activate RNAi response (Hammond *et al*., 2000). Geminivirus like viruses which have ssDNA genome replicate through rolling circle mechanism and forms dsRNA structure by which they induce RNA silencing in plants. Viral vector with specific genes can be use to induce RNA gene silencing or PTGS in many plants (Covey *et al*., 1997).

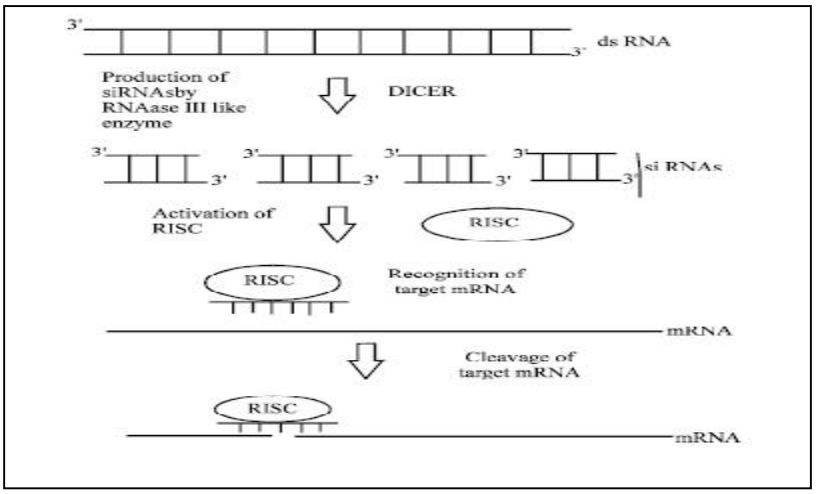

FIGURE 1: General mechanism of RNAi

BEGOMOVIRUS

Begomovirus is the largest genus of family *Geminiviridae*, which is responsible for the losses of crops worldwide. They are known to induce a diversity of symptoms in the plants they infect. However, leaf curling and other leaf distortions seem to be the most frequent symptoms associated with infections by *begomovirus* (Harrison and Robinson, 1999). Sever leaf curling symptoms were observed in recent years but no virus associated with such symptoms was reported (Tiendrebeogo *et al*., 2008). The high rate of symptoms severity on the plant in different region can be demonstrated by susceptibility of some cultivars in the proposed fields (Adjata *et al*., 2008). The virus is transmitted by white fly (*Bemisia tabaci*) and infects dicots. Begomoviruses contain genome either mono-partite or bipartite (Briddon *et al*., 1989). Bipartite begomoviruses contains two molecules of small single strandard circular DNA *i.e*., DNA-A and DNA-B which are same in size of 2.7 kb. These molecules are similar only in the Common Region (CR) of 200 nucleotide (Fauquet and Mayo, 2001). CR has significance in replication and transcription. CR is the site of both 1st and 2nd strand synthesis (Arguello-Astorga *et al*., 1994).

DNA-A has five ORFs (Rep, TrAp, REn, AC_4 and CP) which is responsible for replication and encapsidation (Elmer *et al*., 1988) whereas DNA-B has two ORFs (MP and NSP) which controls the movement in the host (Fig. 2) (Noueiry *et al*., 1994). Generally, monopartite begomo viruses is associated with a satellite DNA (β DNA) which is responsible for the symptom development (Saunders *et al*., 2000; Gaur and Rathore, 2009). All the geminiviruses replicate through Rolling Circle Replication (RCR). The organizations of genome and gene function are conserved in the begomoviruses (Rojas *et al*., 1998).

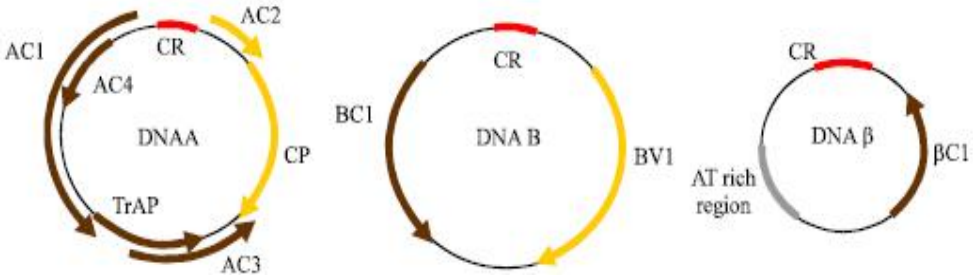

FIGURE 2: Typical genome organization of begomovirus

In the initial stage, the ssDNA transported to the cell nucleus where it replicates through rolling circle mechanism. After replication, CP binds to replicated ssDNA in nucleus and makes a virion particle. Than it translocate to the cytoplasm for systemic movement. MP and NSP protein of DNA-B helps in the systemic infection of the virus (Pinner *et al*., 1993).

RNAi: NATURAL MECHANISM AGAINST VIRAL INFECTION

There are different studies which prove the RNAi mediated resistance against different begomoviruses. This strategy gives a high frequency of resistant plants. There are three ways identified for the gene silencing: cytoplasmic short interfering (siRNA) silencing, silencing of endogenous mRNAs by microRNAs (miRNAs) and DNA methylation and suppression of transcription (Baulcombe, 2004). Cytoplasmic siRNA silencing occurs in response to a viral infection in plants (Ahlquist, 2002). Genetic engineering of sense and antisense RNA in trangenics used effectively against Tomato golden mosaic virus (TGMV) (Day *et al*., 1991). It has been reported that Tomato yellow leaf curl Sardinia virus (TYLCSV), a monopartite begomovirus (Lucioli *et al*., 2003) and

cassava infecting bipartite begomovirus namely *Indian Cassava Mosaic Virus* (ICMV) triggers the PTGS system in infected plants (Mendez-Lozano, *et al*., 2003) with the accumulation of virus specific siRNAs where TYLCSV related siRNAs are specific to viral rep and C4 genes (Lucioli *et al*., 2003). Another bipartite member of begomovirus genus *Pepper golden mosaic virus* (PepGMV) infects dicotyledonous crops like pepper, tomato, tomatillo and tobacco (Mendez-Lozano *et al*., 2003). PepGMV-infected pepper plants show a recovery phenotype followed by the presence of virus-specific siRNAs (Carrillo-Tripp *et al*., 2007). In this way, it's an interesting system to study the gene silencing in viral infected plants. In contrast, some begomoviruses have only a single genomic component which resembles DNA A, such as isolates of *Tomato yellow leaf curl virus* (TYLCV), *Tomato leaf curl virus* (TLCV), *Ageratum yellow vein virus* (AYVV) and *Cotton leaf curl virus* (CLCuV) (Kheyr-Pour *et al.,* 1991; Navot *et al.,* 1991; Dry *et al.,* 1993; Tan *et al.,* 1995; Briddon *et al.,* 2000). There is a new upcoming group of monopartite begomovirus which causes infection in Okra (*Abelmoschus esculentus*) and leads to the economical losses. *Bhendi Yellow Vein Mosaic Virus* (BYVMV) is a complex monopartite begomovirus which requires satellite DNA component for the production of typical symptoms (Jose and Usha, 2003).

BHENDI

Bhendi (*Abelmoschous esculentus* (L.) Moench), also called as Okra or Lady's finger belongs to a family Malvaceae, is one of the important vegetable crops grown in tropical, subtropical and warm sections of the temperate zones of the world (Charrier, 1984). Bhendi can be grown under irrigated conditions, warm moist or hot summer season, and light type of soils with fairely high organic matter content. It is grown commercially in India, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Burma, Japan, Malayasia, Brazil, Ghana, Ethiopian, Cyrpus and the Southern United States. Bhendi is known to be native of South Africa and has been predominantly grown as a vegetable crop of the tropics (Thompson and Kelly, 1957). Originally, it thought to have been present in Africa and India as a polyphytic species, because its semi wild ancestor *Abelmoschus tuberculatus*, occurs in India (Grubben, 1977).

India ranks first in the world with 3.5 million tonnes (70% of the total world production) of okra produced from over 0.35 million ha land (FAOSTAT, 2008). Okra is known by many local names in different parts of the world. It is called lady's finger in England, gumbo in the United States of America, guino-gombo in Spanish, guibeiro in Portuguese and bhindi in India. It is quite popular in India because of easy cultivation, dependable yield and adaptability to varying moisture conditions. Even within India, different names have been given in different regional languages (Chauhan, 1972).

Okra has several uses, its tender fruits are used as vegetable, eaten boiled or in culinary preparations as sliced and fried pieces. Its stem is also used for paper making in paper mills. It is also used in thickening of soups and gravies because of its high mucilage content. Okra fruits are also sliced and sun-dried or canned and pickled for off-season use. Mucilaginous extracts of the green stem are commonly used for clarifying sugarcane juice and in 'gur' industry. Sometimes the seeds are roasted and used as a substitute for coffee (Martin, 1982). Bhendi has good nutritional value, particularly vitamin C (30 mg/100 mg), Calcium (90 mg/100mg) and Iron (1.5 mg/100 mg). India is the largest producer of bhendi, covering an area of 3.98 lakh ha with an annual production of 40.99 lakh tones (Anonymous, 2007).

VIRAL DISEASES OF BHENDI

Despite being an important Indian vegetable crop that is grown extensively throughout the year in all parts of the country, bhendi yields are quite low due to infection by a number of diseases, of which viral diseases are particularly important (Usha, 1980). The viruses reported to cause diseases in okra are yellow vein mosaic (Kulkarni, 1924), enation leaf curl (Singh and Dutta, 1986), okra leaf curl and okra mosaic (Lana, 1976). Among these viral diseases, Yellow vein mosaic disease of bhendi (YVMD) (Fig. 3), characterized by veinal clearing, chlorosis and swelling coupled with slight downward curling of leaf margins, twisting of petioles, and retardation of growth (Capoor and varma, 1950) has been consistently reported in past few years from different parts of India. Up to 96% loss in yield has been reported (Pun and Doraiswamy, 1999). BYVMV (Brunt *et al.,* 1996) was reported from Bombay in India (Kulkarni, 1924). This is the earliest report of this virus, implying that BYVMV might have originated in India. It has been shown to be a geminivirus based on its morphology and its serological relationship with *African cassava mosaic virus* (Harrison *et al.,* 1991).

FIGURE 3: Symptoms of yellow mosaic and vein thickening in okra

OCCURRENCE AND LOSSES

Jha and Mishra (1955) reported that the BYVMV in Bihar was particularly severe in vegetable belts where the crop was almost continuously cultivated throughout the year. The % of infection was from 50-90%. Studies on the effect of BYVMV infection on the growth and yield of Bhendi were conducted by Sastry and Singh (1973) and reported that the growth of the plants was very much stunted when plants were infected at the early stage of the crop. The estimated loss in yield was 93.8 % in 35 days old crop, whereas it was 83.63 % in 70 days old crop. The estimated losses due to BYVMV infection at 30, 45 and 60 days after sowing were 76.0, 54.9 and 47.8 %, whereas the fruit number was reduced to 4, 7 and 8 fruits respectively as compared to 16 fruits in the healthy plants. The corresponding yields were 27, 62 and 94 g/plant, respectively as compared to 222 g/plant in the healthy plants (Chellaiah and Murugesan, 1976). Sinha and Chakrabarti (1978) studied the effect of BYVMV when okra plants were infected at various growth stages and reported that the virus had an adverse effect on plant height, number of branches, number and size of fruits and seed yield. Chaudhary *et al.*, (1995) reported that the incidence of Bhendi yellow vein mosaic virus on okra ranged from 19.26 to 69.23%. Khan and Mukhopadhaya (1985) conducted the gradient study on the spread of bhendi YVMV and showed a steep rise during the early growth stages of the crop. The extent of final infection was dependent on the degree of initial infection.

Nath and Saikia (1995) observed a relationship between crop age and losses in okra due to BYVMV. Maximum (94.42%) and minimum (32.65%) yield losses were recorded for infected plants at 35 and 63 days after sowing, respectively. They suggested that early infection caused heavy yield reductions compared to late infection. Losses could be reduced by controlling spread of the disease by controlling the vector (*Bemisia tabaci*). Ahmed and Patil (2004) conducted a survey on the incidence of Bhendi yellow vein mosaic virus on different okra cultivars during August in Kharif 1999-2000 and during February to March in summer 2000-01 in the districts of Dharwad, Belgaum, Haveri and Gadag. During Kharif, it was 15.08 % and in summer, it was 58.14 % and Arka Anamika showed the lowest disease incidence during Kharif (0.07%) and summer (20.47%).

The rise in importance of this disease has emphasized the need, to properly determine its etiology. Although an earlier report has indicated that YVMD is possibly caused by betasatellite associated monopartite begomoviruses (Jose and Usha, 2003), the begomoviruses associated with YVMD of bhendi across large parts of northern India have never been properly characterized. PCR-amplification using begomovirus-specific primers showed the presence of a begomovirus component equivalent to DNA A in diseased bhendi plants (Jose and Usha, 2000). Rigorous attempts to amplify a second genomic component (DNA B) were not successful, suggesting that BYVMV is a monopartite begomovirus. Cataloging the full complement of species associated with a disease such as YVMD is important, because in the past few years it has become apparent that in many parts of the world such disease are in fact caused by a diverse complex of different

begomovirus species (Briddon *et al*., 2001; Bull *et al*., 2006; Lefeuvre *et al*., 2007; Zhou *et al*., 2008). Such complexes and the opportunities for mixed infections that afford a crucial feature of begomovirus ecology for the inter-species recombination seems to be a major driver of begomovirus evolution. It was quite probable that, recombination during the last 50 years has directly contributed to the emergence of multiple new begomovirus diseases (Berrie *et al*., 2001; Jeske *et al*., 2001; Padidam *et al*., 1999; Schnippenkoetter *et al*., 2001). There is requirement of such resistance system which can work against the broad range of viruses. In this case, the PTGS is most reliable strategy because its RNA mediated and induces resistance against invading virus. An approach to target Rep and AV_2 gene by antisense expression is more successful (Sanjaya *et al*., 2005). RNAi involved resistance against DNA virus also reported in Cassava. Many copies of AC_1 gene of ACMV found to be accumulated in plants. These siRNAs are effective against ACMV and two other geminiviruses (Chellappan *et al*., 2004). The AC_1 sequence is conserved (66-77%) among geminiviruses through which RNA silencing proved to be an effective strategy to produce stable and broad geminivirus resistance. Similarly, RNA induced gene silencing was studied with TYLCV. Non coding conserved region were taken from the different geminiviruses and used to make construct and targeted for the broad resistance against tomato leaf curl viral disease (Abhary *et al*., 2006). Golden mosaic disease of *Phaseolus vulgaris* is caused by BGMV (Bean golden mosaic virus). This is the largest constraints in bean production. Many strategies of genetic engineering are employed for the resistance of this virus. In which production of truncated defective gene (Antignus *et al*., 2004) and antisense RNA is included (Asad *et al*., 2003).

In 2002, Dharmacon was the first company to develop an algorithm for the rational design for highly potent siRNA gene silencing tool. The initial data for this discovery was published in Cell and Nature Biotechnology (Khvorova *et al*., 2003; Reynolds *et al*., 2004). Now there are several companies that have developed computer algorithms for analysis of a gene sequence based on a number of parameters and predict the most effective siRNA sequences for silencing that particular gene. There are several free software programs (applications) available on the internet for selecting the most effective region to be used for RNAi.

There are several interactive resources available on the internet free to use by any researchers. One would benefit by learning more about each software application and use the right tool to design silencing RNA fragment for a particular gene of interest. The web sites containing such RNAi related software applications are listed here.

- Silencing RNA Target Finder from Ambion **http://www.ambion.com/techlib/misc/siRNA_finder.html**

- RNAi Design Tool from Integrated DNA Technologies
  http://biotools.idtdna.com/rnai/
- Gene Specific siRNA Selector
  http://hydra1.wistar.upenn.edu/Projects/siRNA/siRNAindex.htm
- RNAi Designer from Invitrogen
  https://rnaidesigner.invitrogen.com/sirna/
- siRNA Selection Program, Whitehead Institute for Biomedical Research.
  http://jura.wi.mit.edu/siRNAext/register.php
- Summary of siRNA Design Rules and Tools
  http://www.protocolonline.org/prot/Molecular_Biology/RNA/RNA_Interference/siRNA_Design_Rules
- General Resources on RNAi in Ambion
  http://www.ambion.com/techlib/resources/RNAi/

RNAi AS A POTENT TOOL

The basic use of RNAi is to produce virus resistant crops. This natural process is interesting because it provides limits the viral infection (Voinnet and Baulcomb, 1997). Initially its importance was not recognized due to a misconception that it is limited to petunia with few other species (Cogoni and Macino, 1999). Nowadays it's the hot topic of molecular biology. RNAi is a favorable tool to knock down or silence a gene expression because it can target multiple gene family member by same RNAi inducing transgene. Another advantage of RNAi is related to its dominant aspect. It can knock down genes in polyploidy genome which contain four or more orthologs. Recent methods for RNAi silencing in plants are based upon an inverted repeat mRNA expression (Waterhouse *et al*., 1998). The best parts of these methods are that they are useful in both stable transformation and transient transfection. Few studies suggested an approach to improve the efficiency of silencing by the insertion of an intron between the inverted flanking target sequences (Smith and Gross, 2000). These RNA silencing strategies are suitable for crop improvement by reduce infection of RNA or DNA virus. Many plant viruse have ssRNA genome, which can deliver gene in PTGS system by inoculation of viral vector transcripts. In the case of DNA virus like geminivirus it becomes easier because it need only the viral DNA. Except this, cDNA of viral genome can be introduced in plant cell with the binary vector. There are several traditional gene knock-out methods which require transformation and tissue culture but PTGS is rapid and more effective.

RNA gene silencing is the mechanism which is conserved across the kingdoms. Interesting feature of geminiviruses is that they induce gene silencing without having dsRNA phase in their replication. They activate host PTGS system by the siRNAs which are specific for viruses. Geminiviruses encode AC2 and AC4 PTGS suppressor proteins that indicate the different evolution of this virus in respect to interaction with the host. This has been clear with the several studies that the geminiviruses and their statellite DNA are very useful for the gene silencing in various plants and also in post-genomic studies. Though a lot of studies have been done on RNAi mechanism related to geminiviruses but still many questions are there to be answered. As geminiviruse does not have any dsRNA structure in their replication cycle then how do they induce RNA silencing. There are some unproved theories which supports it, such as presence of overlapping transcripts and the presence of several mRNAs but no authenticated proof. How PTGS is controlled by suppressor proteins. Is there any inter-action of these suppressor proteins with miRNA pathway? The answers of these questions can help us to understand PTGS more precisely. As the new geminiviruses and their satellite DNAs are emerging rapidly, they can be devastating for the crops. To improve food production it must be controlled. For this we need to identify the common region of viral sequences to target a broad range of viruses and their PTGS suppressor system. RNAi or PTGS can be utilize as a potential tool against emerging geminiviruses to protect the crops. In respect to the present researches on RNAi can be considered as revolution in plant biotechnology and an effective tool in functional genomics. It's a broad application of biotechnology from molecular biology to gene therapy. Virus induced gene silencing has an enormous power to down regulate the endogenous genes that are virus-specific.

CONCLUSION

Bhendi yellow vein mosaic disease is the worst virus considered in the world which causes 50-90% crop losses. There are approximately 60 forms of viruses which affect crops. Current agricultural technology needs more and more molecular tools to reduce current crop loss and feed extra mouths, which according to a recent estimate by the FAO (Food and Agriculture Organization) will increase by two billion over the next 30 years. RNAi is the favorable tool to fight against this emerging threat of geminiviruses. RNAi is the pathway for silencing of whole gene families or unrelated genes through targeting of specific gene sequences or by using a single RNAi construct which contains several target sequencing. If judiciously used, this technology may go a long way to narrow the gap through production of disease, insect and virus resistant, nutritionally rich and toxic-free crops. Since this technology offers a great potential in understanding gene functions and utilize them to improve crop quality and production. Although the basic concept of the application of transgene-based RNAi to the genetic improvement of crop plants has been established, further feasibility studies are needed for its wider application.

REFERENCES

Abhary, M.K., Anfoka, G.H., Nakhla, M.K. and Maxwell, D.P. (2006) Post-transcriptional gene silencing in controlling viruses of the tomato yellow leaf curl virus complex. Arch. Virol., 151: 2349-2363.

Adjata, K.D., Muller, E., Aziadekey, M., Gumedzoe Y.M.D. & Peterschmitt, M. (2008) Incidence of cassava viral diseases and first identification of East African cassava mosaic virus and Indian cassava mosaic virus by PCR in Cassava (*Manihot esculenta* Crantz) fields in Togo. Am. J. Plant Physiol., **3:** 73-80.

Ahlquist, P. (2002) RNA-dependent RNA polymerases, viruses and RNA silencing. Science, 296: 1270-1273.

Ahmed, Z. & Patil, M. S. (2004) Incidence of yellow vein mosaic virus on different okra cultivars in Karnataka. J. Agric. Sci., 17(3): 615-616.

Anonymous (2007) Area and production of horticulture crops during 2005-06 to 2007-08. Ministry of Agriculture, India.(www.dacnet.nic.in/eands/At_Glance2008/ hpcrops).

Antignus, Y., Vunsh, R., Lachman, O., Pearlsman, M., Maslenin, L., Hananya, U. & Rosner, A. (2004) Truncated rep gene originated from Tomato yellow leaf curl virus-Israel (Mild) confers strain-specific resistance in transgenic tomato. Ann. Applied Biol., 144: 39-44.

Arguello-Astorga, G.R., Guevara-Gonzalez, R.G., Herrera-Estrella, L.R. & Rivera-Bustamante, R.F. (1994) Geminivirus replication has a group specific organisation of iterative elements: A model for replication. Virology, 203: 90-100.

Asad, S., Haris, W.A., Bashir, A., Zafar, Y., Malik, K.A. Malik, N.N. and Lichtenstein, C.P. (2003) Transgenic tobacco expressing geminiviral RNAs are resistant to the serious viral pathogen causing cotton leaf curl disease. Arch. Virol., 148: 2341-2352.

Bass, B.L. (2000) Double-stranded RNA as a template for gene silencing. Cell, 101: 235-238.

Baulcombe, D. (2004) RNA silencing in plants. Nature, 431**:** 356-363.

Berrie, L.C., Rybicki, E.P., Rey, M.E.C. (2001) Complete nucleotide sequence and host range of South African cassava mosaic virus: further evidence for recombination amongst begomoviruses. J. Gen. Virol., 82: 53-58.

Briddon, R.W., Watts, J., Markham, P.G. and Stanley, J. (1989) The coat protein of beet curly top virus is essential for infectivity. Virology, 172: 628-633.

Briddon, R.W., Mansoor, S., Bedford, I.D., Pinner, M.S. and Markham, P.G. (2000) Clones of cotton leaf curl geminivirus induce symptoms atypical of cotton leaf curl disease. Virus Genes, 20: 19-26.

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

Brunt, A.A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J. and Watson, L. (1996) Viruses of Plants-Description and lists from the VIDE Database. CAB International (Wallingford, UK). pp. 233-234.

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. J. Gen. Virol., 87**:** 3053-3065.

Capoor, S.P. & Varma, P. M. (1950) Yellow vein mosaic of *H. esculentus*. L. Indian J. Agric. Sci., 20: 217-230.

Carrillo-Tripp, J., Lozoya-Gloria, E. and Rivera-Bustamante, R.F. (2007) Symptom remission and specific resistance of pepper plants after infection by Pepper golden mosaic virus. Phytopathology, 97: 51-59.

Charrier, A. (1984) Genetic resources of the genus *Abelmoschus*. International Board for Plant Genetic Resources, Rome, pp. 21-41.

Chaudhary, D.R., Vidyasagar & Jagmohan, K. (1995) A note on the occurrence of yellow vein mosaic in intervarietal crosses of okra. Himachal Pradesh. J. Agric. Res., 21**:** 90-92.

Chauhan, D.V.S. (1972) Vegetable Production in India, 3rd ed., Ram Prasad and Sons (Agra).

Chellaiah, S. & Murugesan, S. (1976) Estimation of loss due to yellow vein mosaic disease in Bhendi. Madras Agric.J., 63: 169-170.

Chellappan, P., Vanitharani, R. and Fauquet, C.M. (2004) Short Interfering RNA Accumulation Correlates with Host Recovery in DNA Virus-Infected Hosts, and Gene Silencing Targets Specific Viral Sequences. J. Virol., 78**:** 7465-7477.

Cogoni, C. & Macino, G. (1999) Homology dependent gene silencing in plants and fungi: A number of variations on the same theme. Curr. Opin. Microbiol., 2**:** 657-662.

Covey, S. N., Al-Kaff, N. S., Langara, A. & Turner, D. S. (1997) Plants combat infection by gene silencing. **Nature, 385:** 781-782.

Day, A.G., Bejarano, E.R., Buck, K.W., Burrell, M. & Lichtenstein, C. P. (1991) Expression of an antisense viral gene in transgenic tobacco confers resistance to the DNA virus tomato golden mosaic virus. **Proc. Natl Acad. Sci. USA., 88:** 6721-6725.

Dry, I.B., Rigden, J.E., Krake, L.R., Mullineaux, P.M. & Rezaian, M.A. (1993) Nucleotide sequence and genome organization of tomato leaf curl geminivirus. J. Gen. Virol., 74**:** 147-151.

Elmer, J.S., Brand, L., Sunter, G., Gardiner, W.E., Bisaro, D.M. & Rogers, S.G. (1988) Genetic analysis of tomato golden mosaic virus II. The product of the AL1 coding sequence is required for replication. Nucl. Acid Res., 16: 7043-7060.

FAOSTAT. 2008. (http://www.fao.org)

Fauquet, C.M. & Mayo, M. A. (2001) The 7th ICTV report. Arch. Virol., 146: 189-194.

Finnegan, E.J., Margis, R. & Waterhouse, P.M. (2003) Posttranscriptional gene silencing is not compromised in the *Arabidopsis* CARPEL FACTORY (DICER-LIKE1) mutant ◆ a homolog of DICER-1 from Drosophila. Curr. Biol., 13**:** 236-240.

Gaur, R.K. & Rathore, M.S. (2009) RNAi mediated gene silencing against begomovirus in India. Project submitted to Department of Biotechnology, Govt. of India.

Grubben, G.S.H. (1977) Tropical agriculture and their genetic resources. FAO, Rome, p.120.

Hamilton, A. & Baulcombe, D.C. (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. Science, 286: 950-952.

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

Harrison, B.D. and Robinson, D.J. (1999) Natural genomic and antigenic variation in whitefly- transmitted Geminiviruses (Begomoviruses). Annu. Rev. Phytopathol., 37**:** 369-398.

Harrison, B.D., Muniyappa, V., Swanson, M.M., Roberts I.M. and Robinson, D.J. (1991) Recognition and differentiation of seven whitefly transmitted geminiviruses from India and their relationships to African cassava mosaic and Thailand mungbean yellow mosaic viruses. Annl. Appl. Biol., 118: 299-308.

Jeske, H., Lutgemeier, M., Preiss, W. (2001) DNA forms indicate rolling circle and recombination-dependent replication of Abutilon mosaic virus. EMBO.J., 20: 6158-6167.

Jha, A. & Mishra, J.N. (1955) Yellow vein mosaic of bhendi in Bihar. In: Proc. Bihar Acad. Agric. Sci., 4: 129-130.

Jose, J. & Usha, R. (2000) Extraction of geminiviral DNA from a highly mucilaginous plant (*Abelmoschus esculentus*). Plant Mol. Biol. Reptr., 18: 349-355.

Jose, J. & Usha, R. (2003) Bhendi yellow vein mosaic disease in India is caused by association of a DNA beta statellite with a begomovirus. Virology, 305: 310-317.

Khan, M. A. & Mukhopadhyay, S. (1985) Studies on the seasonal spread of yellow vein mosaic virus disease of okra. Indian Phytopath., **38:** 688-691.

Kheyr-Pour, A., Bendahmane, M., Matzeit, V., Accoto, G.P.M., Crespi, S. & Gronenborn, B. (1991) Tomato yellow leaf curl virus from Sardinia is a whitefly-transmitted monopartite geminivirus. Nucleic Acids Res., 19**:** 6763-6769.

Khvorova, A., Reynolds, A. & Jayasena, S.D. (2003) Functional siRNAs and rniRNAs exhibit strand bias. Cell, 115: 209-216.

Kulakarni, G. S. (1924) Mosaic and other related diseases of crops in the Bombay presidency. Poona Agricultural College Magazine, 16: 6-12.

Lana, A.F. (1976) Mosaic virus and leaf curl diseases of Okra in Nigeria. PANS (Pest Articles and News Summaries), **22:** 474-478.

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. J. Gen. Virol., 88: 3458–3468.

Lucioli, A., Noris, E., Brunetti, A., Tavazza, R. and Ruzza, V. (2003) Tomato yellow leaf curl Sardinia virus rep-derived resistance to homologous and heterologous geminiviruses occurs by different mechanisms and is overcome if virus-mediated transgene silencing is activated. J. Virol., 77: 6785-6798.

Martin, F. W. 1982. A second edible okra species and its hybrids with common okra. **Ann. Bot., 50:** 277-283.

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

Napoli, C., Lemiex, C. & Jorgensen, R. (1990) Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in Trans. Plant Cell, 2: 279-289.

Nath, P. & Saikia, A.K. (1995) Influence of sowing time on yellow vein mosaic virus on Ahmed, Z. and Patil, M. S., 2004, Incidence of yellow vein mosaic virus on different okra cultivars in Karnataka. J. Agric. Sci., 17(3): 615-616.

Navot, N., Pichersky, E., Zeidan, M., Zamir, D. & Czosnek, H. (1991) Tomato yellow leaf curl virus: A whitefly-transmitted geminivirus with a single genomic component. Virology, 185: 151-161.

Noueiry, A. O., Luces, W. J. & Gillbertson, R. L. (1994) Two proteins of a plant DNA virus co-ordinate nuclear and plasmodesmal transport. **Cell, 76:** 925-932.

Padidam, M., Sawyer, S., Fauquet, C.M. (1999) Possible emergence of new geminiviruses by frequent recombination. Virology, 265: 218-225.

Pinner, M.S., Medina, V., Plaskitt, K.A. & Markham, P. G. (1993) Viral inclusions in monocotyledons infected by maize streak and related geminiviruses. Plant pathol., 42: 75-87.

Pun, K.B. & Sabitha Doraiswamy (1999) Effect of age of okra plants on susceptibility to Okra yellow vein mosaic virus. Indian J. Virol., 15: 57-58.

Usha, R. (1980) In: Characterization, diagnosis & management of plant viruses, ed. by G.P. Rao, P.L. Kumar, R.L. Holguın-Pena (Studium Press, Houston,), pp. 387-392.

Reynolds, A., Leake, D., Boese, Q., Scaringe, S., Marshall, W. S. and Khvorova, A. (2004) Rational siRNA design for RNA interference. Nature Biotechnology, 22**:** 326-330.

Rojas, M.R., Noueiry, A.O., Lucas, W.J. & R. Gillbertson, L. (1998) Bean dwarf mosaic geminivirus movement proteins recognize DNA in a form and size specific manner. Cell, 95: 105-113.

Sanjaya, V.V.S., Prasad, V., Kirthi, N., Maiya, S.P., Savithri, H.S. and Sita, G.L. (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.

Sastry, K.S.M. & Singh, S.J. (1973) Restriction of yellow vein mosaic spread in okra through the control of vector, *Bemisia tabaci*. Indian J. Mycol. Pl. Pathol., 3**:** 76-80.

Saunders, K., Bedford, I.D., Briddon, R.W., Markham, P.G., Wong, S.M. and Stanley, J. (2000) A unique virus complex causes Ageratum yellow vein disease. Proc. Nat. Acad. Sci., 97**:** 6890-6895.

Schnippenkoetter, W.H., Martin, D.P., Willment, J.A., E. Rybicki, P. ((2001) Complete nucleotide sequence and host range of South African cassava mosaic virus: further evidence for recombination amongst begomoviruses. J. Gen. Virol., 82: 3081-3090.

Singh, S. J. & Dutta, O. P. (1986) Enation leaf curl virus of okra-a new virus disease. Indian J. Virol., 2(1): 114-117.

Sinha, S.N. and Chakrabarthi, A.K. (1978) Effect of yellow vein mosaic virus infection on okra seed production. Seed Res., 6: 67-70.

Smith, D.L. & Gross, K.C. (2000) A family of at least seven beta-galactosidase genes is expressed during tomato fruit development. Plant Physiol., 123**:** 1173-1183.

Tan, P.H.N., Wong, S.M., Wu, M., Bedford, I.D., Saunders, K. and Stanley, J. (1995) Genome organization of Ageratum yellow vein virus, a monopartite whitefly-transmitted geminivirus isolated from a common weed. J. Gen. Virol., 76: 2915-2922.

Thompson, C. H. & Kelly, C.W. (1957) Vegetable crops, Mcgraw Hill Book, Co. Inc., USA.

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

Voinnet, O. & Baulcomb, D.C. (1997) Systemic silencing in gene signalling. Nature, 389: 553-553.

Waterhouse, P.M., Graham, M.W. & Wang, M.B. (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, Z., Johansen, L.K., Gustafson, A.M., Kasschau, K.D. & Lellis, A.D. (2004) Genetic and functional diversification of small RNA pathways in plants. PLoS Biol., 2: E104-E104.

Zhou, Y.C., Nonssourou, M., Kon, M. R. Rajas, H. Jiang, Chen, L.F., Gamby, K., Foster, R., Gilbertson, R. L. (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. Arch. Virol., 153: 693-706.

GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

Review Article

DEVELOPING RNAi STRATEGY AGAINST YELLOW VEIN MOSAIC DISEASE (YMVD) OF OKRA

*Geetha Shanmugam, Kumar Krish & Balasubramanian Ponnuswami
Department of Plant Biotechnology, Centre for plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641003 (Tamil Nadu), INDIA
*Corresponding author email: geethshan@gmail.com

ABSTRACT
Okra (*Abelmoschus esculentus*; family *Malvaceae*) is grown in temperate as well as subtropical regions of the world, both for human consumption as a vegetable and for industrial uses. Okra yields are affected by the diseases caused by phytopathogenic viruses. India is the largest producer of okra and in this region a major biotic constraint to production are viruses of the genus *Begomovirus*. Yellow vein mosaic disease (YVMD) of bhendi is spreading rapidly throughout India, affecting plants at all growth stages, and resulting in plants yielding unmarketable fruits and clearly a most serious of the newly emerging/reemerging begomovirus-associated diseases in India. Despite its importance, the etiology of YVMD across much of India is unknown and has been shown to be caused by beta satellite associated monopartite begomovirus species. A rich diversity of viruses infecting Indian bhendi is of concern, since this situation undoubtedly increases incidences of mixed infections and increases the possibility of yet more novel recombinant viruses arising within this species. Together with the already existing diversity of begomoviruses infecting bhendi, such novel recombinants will probably further undermine efforts to control the virus using transgenic or inbred resistance strategies. Gene silencing can occur either repression of transcription, termed Transcriptional Gene Silencing (TGS) or through mRNA degradation, termed post transcriptional gene silencing. Transcriptional gene silencing results from a marked decrease in transcription and hypermethylaton of genes affected. RNAi or PTGS discovered as a natural anti-viral system in plants. Studies revealed that geminiviruses which replicates in nucleus can induce PTGS and become the target for it. RNAi has a strong potential to reduce the infection of geminiviruses. Several studies are already conducted on different genes geminiviruses and that are used for generating virus resistance plants. Our main objective of present study is to develop resistance against geminivirus using a novel strategy based on RNAi. Here we summarise how the RNAi mechanism works against begomovirus infection in plants and how we can utilize it to reduce the losses.

KEYWORDS: RNAi, Okra, Yellow vein mosaic disease, *Geminivirus*, *Begomovirus*, Beta satellite.

INTRODUCTION

RNAi also known as RNA silencing and PTGS (Post transcriptional gene silencing), a natural mechanism works against viral infection in both plants and animals (Napoli *et al*., 1990). It is targeted by dsRNA which leads to the degradation of sequence specific viral RNA (Fig. 1) (Hamilton and Baulcombe, 1999). Double strandard RNAs generated by virus are recognized and cleaved into small interfering RNAs of approximately 25 nucleotide by the RNAase III like enzymes (Bass, 2000) called as DICER. There are four dicer like enzymes identified in plants. First involved in miRNA biogenesis (Finnegan *et al*., 2003), second play role in viral siRNA production and third is responsible for transposon siRNA production (Xie *et al*., 2004). These siRNAs incorporate into the RISC (RNA induced silencing complex) and guide the degradation of viral RNA. Many viruses having ssRNA genome which released from the protein coat when they enter a cell. They replicate through virus encoded RNA-dependent RNA polymerase and generate sense and antisense RNA. These RNAs forms dsRNA and activate RNAi response (Hammond *et al*., 2000). Geminivirus like viruses which have ssDNA genome replicate through rolling circle mechanism and forms dsRNA structure by which they induce RNA silencing in plants. Viral vector with specific genes can be use to induce RNA gene silencing or PTGS in many plants (Covey *et al*., 1997).

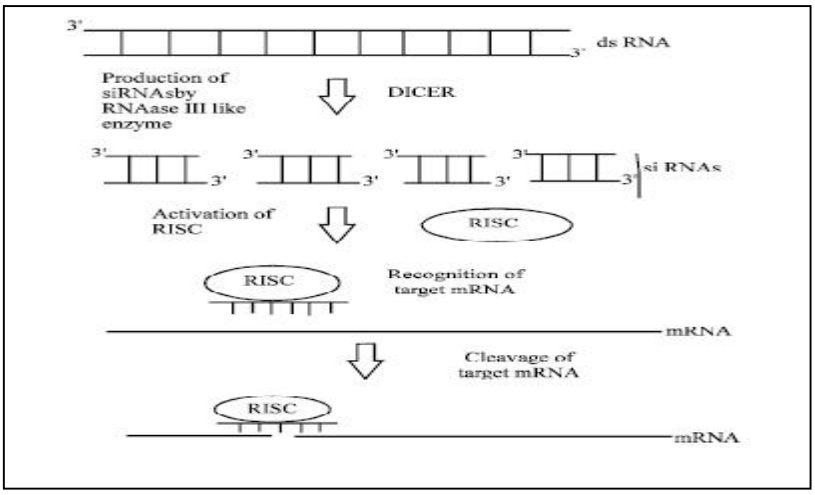

FIGURE 1: General mechanism of RNAi

BEGOMOVIRUS

Begomovirus is the largest genus of family *Geminiviridae*, which is responsible for the losses of crops worldwide. They are known to induce a diversity of symptoms in the plants they infect. However, leaf curling and other leaf distortions seem to be the most frequent symptoms associated with infections by *begomovirus* (Harrison and Robinson, 1999). Sever leaf curling symptoms were observed in recent years but no virus associated with such symptoms was reported (Tiendrebeogo *et al*., 2008). The high rate of symptoms severity on the plant in different region can be demonstrated by susceptibility of some cultivars in the proposed fields (Adjata *et al*., 2008). The virus is transmitted by white fly (*Bemisia tabaci*) and infects dicots. Begomoviruses contain genome either mono-partite or bipartite (Briddon *et al*., 1989). Bipartite begomoviruses contains two molecules of small single strandard circular DNA *i.e*., DNA-A and DNA-B which are same in size of 2.7 kb. These molecules are similar only in the Common Region (CR) of 200 nucleotide (Fauquet and Mayo, 2001). CR has significance in replication and transcription. CR is the site of both 1st and 2nd strand synthesis (Arguello-Astorga *et al*., 1994).

DNA-A has five ORFs (Rep, TrAp, REn, AC_4 and CP) which is responsible for replication and encapsidation (Elmer *et al*., 1988) whereas DNA-B has two ORFs (MP and NSP) which controls the movement in the host (Fig. 2) (Noueiry *et al*., 1994). Generally, monopartite begomo viruses is associated with a satellite DNA (β DNA) which is responsible for the symptom development (Saunders *et al*., 2000; Gaur and Rathore, 2009). All the geminiviruses replicate through Rolling Circle Replication (RCR). The organizations of genome and gene function are conserved in the begomoviruses (Rojas *et al*., 1998).

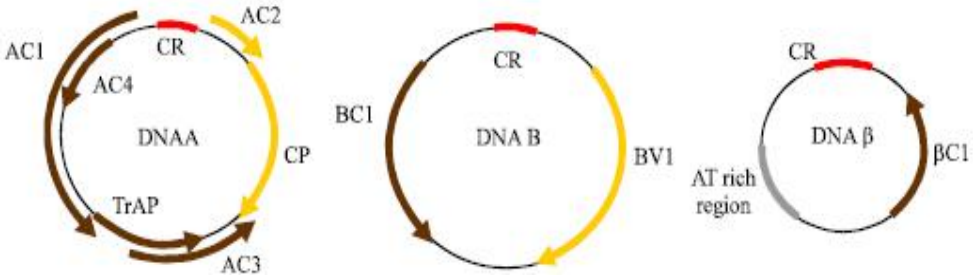

FIGURE 2: Typical genome organization of begomovirus

In the initial stage, the ssDNA transported to the cell nucleus where it replicates through rolling circle mechanism. After replication, CP binds to replicated ssDNA in nucleus and makes a virion particle. Than it translocate to the cytoplasm for systemic movement. MP and NSP protein of DNA-B helps in the systemic infection of the virus (Pinner *et al*., 1993).

RNAi: NATURAL MECHANISM AGAINST VIRAL INFECTION

There are different studies which prove the RNAi mediated resistance against different begomoviruses. This strategy gives a high frequency of resistant plants. There are three ways identified for the gene silencing: cytoplasmic short interfering (siRNA) silencing, silencing of endogenous mRNAs by microRNAs (miRNAs) and DNA methylation and suppression of transcription (Baulcombe, 2004). Cytoplasmic siRNA silencing occurs in response to a viral infection in plants (Ahlquist, 2002). Genetic engineering of sense and antisense RNA in trangenics used effectively against Tomato golden mosaic virus (TGMV) (Day *et al*., 1991). It has been reported that Tomato yellow leaf curl Sardinia virus (TYLCSV), a monopartite begomovirus (Lucioli *et al*., 2003) and

cassava infecting bipartite begomovirus namely *Indian Cassava Mosaic Virus* (ICMV) triggers the PTGS system in infected plants (Mendez-Lozano, *et al*., 2003) with the accumulation of virus specific siRNAs where TYLCSV related siRNAs are specific to viral rep and C4 genes (Lucioli *et al*., 2003). Another bipartite member of begomovirus genus *Pepper golden mosaic virus* (PepGMV) infects dicotyledonous crops like pepper, tomato, tomatillo and tobacco (Mendez-Lozano *et al*., 2003). PepGMV-infected pepper plants show a recovery phenotype followed by the presence of virus-specific siRNAs (Carrillo-Tripp *et al*., 2007). In this way, it's an interesting system to study the gene silencing in viral infected plants. In contrast, some begomoviruses have only a single genomic component which resembles DNA A, such as isolates of *Tomato yellow leaf curl virus* (TYLCV), *Tomato leaf curl virus* (TLCV), *Ageratum yellow vein virus* (AYVV) and *Cotton leaf curl virus* (CLCuV) (Kheyr-Pour *et al.,* 1991; Navot *et al.,* 1991; Dry *et al.,* 1993; Tan *et al.,* 1995; Briddon *et al.,* 2000). There is a new upcoming group of monopartite begomovirus which causes infection in Okra (*Abelmoschus esculentus*) and leads to the economical losses. *Bhendi Yellow Vein Mosaic Virus* (BYVMV) is a complex monopartite begomovirus which requires satellite DNA component for the production of typical symptoms (Jose and Usha, 2003).

BHENDI

Bhendi (*Abelmoschous esculentus* (L.) Moench), also called as Okra or Lady's finger belongs to a family Malvaceae, is one of the important vegetable crops grown in tropical, subtropical and warm sections of the temperate zones of the world (Charrier, 1984). Bhendi can be grown under irrigated conditions, warm moist or hot summer season, and light type of soils with fairely high organic matter content. It is grown commercially in India, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Burma, Japan, Malayasia, Brazil, Ghana, Ethiopian, Cyrpus and the Southern United States. Bhendi is known to be native of South Africa and has been predominantly grown as a vegetable crop of the tropics (Thompson and Kelly, 1957). Originally, it thought to have been present in Africa and India as a polyphytic species, because its semi wild ancestor *Abelmoschus tuberculatus*, occurs in India (Grubben, 1977).

India ranks first in the world with 3.5 million tonnes (70% of the total world production) of okra produced from over 0.35 million ha land (FAOSTAT, 2008). Okra is known by many local names in different parts of the world. It is called lady's finger in England, gumbo in the United States of America, guino-gombo in Spanish, guibeiro in Portuguese and bhindi in India. It is quite popular in India because of easy cultivation, dependable yield and adaptability to varying moisture conditions. Even within India, different names have been given in different regional languages (Chauhan, 1972).

Okra has several uses, its tender fruits are used as vegetable, eaten boiled or in culinary preparations as sliced and fried pieces. Its stem is also used for paper making in paper mills. It is also used in thickening of soups and gravies because of its high mucilage content. Okra fruits are also sliced and sun-dried or canned and pickled for off-season use. Mucilaginous extracts of the green stem are commonly used for clarifying sugarcane juice and in 'gur' industry. Sometimes the seeds are roasted and used as a substitute for coffee (Martin, 1982). Bhendi has good nutritional value, particularly vitamin C (30 mg/100 mg), Calcium (90 mg/100mg) and Iron (1.5 mg/100 mg). India is the largest producer of bhendi, covering an area of 3.98 lakh ha with an annual production of 40.99 lakh tones (Anonymous, 2007).

VIRAL DISEASES OF BHENDI

Despite being an important Indian vegetable crop that is grown extensively throughout the year in all parts of the country, bhendi yields are quite low due to infection by a number of diseases, of which viral diseases are particularly important (Usha, 1980). The viruses reported to cause diseases in okra are yellow vein mosaic (Kulkarni, 1924), enation leaf curl (Singh and Dutta, 1986), okra leaf curl and okra mosaic (Lana, 1976). Among these viral diseases, Yellow vein mosaic disease of bhendi (YVMD) (Fig. 3), characterized by veinal clearing, chlorosis and swelling coupled with slight downward curling of leaf margins, twisting of petioles, and retardation of growth (Capoor and varma, 1950) has been consistently reported in past few years from different parts of India. Up to 96% loss in yield has been reported (Pun and Doraiswamy, 1999). BYVMV (Brunt *et al.,* 1996) was reported from Bombay in India (Kulkarni, 1924). This is the earliest report of this virus, implying that BYVMV might have originated in India. It has been shown to be a geminivirus based on its morphology and its serological relationship with *African cassava mosaic virus* (Harrison *et al.,* 1991).

FIGURE 3: Symptoms of yellow mosaic and vein thickening in okra

OCCURRENCE AND LOSSES

Jha and Mishra (1955) reported that the BYVMV in Bihar was particularly severe in vegetable belts where the crop was almost continuously cultivated throughout the year. The % of infection was from 50-90%. Studies on the effect of BYVMV infection on the growth and yield of Bhendi were conducted by Sastry and Singh (1973) and reported that the growth of the plants was very much stunted when plants were infected at the early stage of the crop. The estimated loss in yield was 93.8 % in 35 days old crop, whereas it was 83.63 % in 70 days old crop. The estimated losses due to BYVMV infection at 30, 45 and 60 days after sowing were 76.0, 54.9 and 47.8 %, whereas the fruit number was reduced to 4, 7 and 8 fruits respectively as compared to 16 fruits in the healthy plants. The corresponding yields were 27, 62 and 94 g/plant, respectively as compared to 222 g/plant in the healthy plants (Chellaiah and Murugesan, 1976). Sinha and Chakrabarti (1978) studied the effect of BYVMV when okra plants were infected at various growth stages and reported that the virus had an adverse effect on plant height, number of branches, number and size of fruits and seed yield. Chaudhary *et al.*, (1995) reported that the incidence of Bhendi yellow vein mosaic virus on okra ranged from 19.26 to 69.23%. Khan and Mukhopadhaya (1985) conducted the gradient study on the spread of bhendi YVMV and showed a steep rise during the early growth stages of the crop. The extent of final infection was dependent on the degree of initial infection.

Nath and Saikia (1995) observed a relationship between crop age and losses in okra due to BYVMV. Maximum (94.42%) and minimum (32.65%) yield losses were recorded for infected plants at 35 and 63 days after sowing, respectively. They suggested that early infection caused heavy yield reductions compared to late infection. Losses could be reduced by controlling spread of the disease by controlling the vector (*Bemisia tabaci*). Ahmed and Patil (2004) conducted a survey on the incidence of Bhendi yellow vein mosaic virus on different okra cultivars during August in Kharif 1999-2000 and during February to March in summer 2000-01 in the districts of Dharwad, Belgaum, Haveri and Gadag. During Kharif, it was 15.08 % and in summer, it was 58.14 % and Arka Anamika showed the lowest disease incidence during Kharif (0.07%) and summer (20.47%).

The rise in importance of this disease has emphasized the need, to properly determine its etiology. Although an earlier report has indicated that YVMD is possibly caused by betasatellite associated monopartite begomoviruses (Jose and Usha, 2003), the begomoviruses associated with YVMD of bhendi across large parts of northern India have never been properly characterized. PCR-amplification using begomovirus-specific primers showed the presence of a begomovirus component equivalent to DNA A in diseased bhendi plants (Jose and Usha, 2000). Rigorous attempts to amplify a second genomic component (DNA B) were not successful, suggesting that BYVMV is a monopartite begomovirus. Cataloging the full complement of species associated with a disease such as YVMD is important, because in the past few years it has become apparent that in many parts of the world such disease are in fact caused by a diverse complex of different

begomovirus species (Briddon *et al*., 2001; Bull *et al*., 2006; Lefeuvre *et al*., 2007; Zhou *et al*., 2008). Such complexes and the opportunities for mixed infections that afford a crucial feature of begomovirus ecology for the inter-species recombination seems to be a major driver of begomovirus evolution. It was quite probable that, recombination during the last 50 years has directly contributed to the emergence of multiple new begomovirus diseases (Berrie *et al*., 2001; Jeske *et al*., 2001; Padidam *et al*., 1999; Schnippenkoetter *et al*., 2001). There is requirement of such resistance system which can work against the broad range of viruses. In this case, the PTGS is most reliable strategy because its RNA mediated and induces resistance against invading virus. An approach to target Rep and AV_2 gene by antisense expression is more successful (Sanjaya *et al*., 2005). RNAi involved resistance against DNA virus also reported in Cassava. Many copies of AC_1 gene of ACMV found to be accumulated in plants. These siRNAs are effective against ACMV and two other geminiviruses (Chellappan *et al*., 2004). The AC_1 sequence is conserved (66-77%) among geminiviruses through which RNA silencing proved to be an effective strategy to produce stable and broad geminivirus resistance. Similarly, RNA induced gene silencing was studied with TYLCV. Non coding conserved region were taken from the different geminiviruses and used to make construct and targeted for the broad resistance against tomato leaf curl viral disease (Abhary *et al*., 2006). Golden mosaic disease of *Phaseolus vulgaris* is caused by BGMV (Bean golden mosaic virus). This is the largest constraints in bean production. Many strategies of genetic engineering are employed for the resistance of this virus. In which production of truncated defective gene (Antignus *et al*., 2004) and antisense RNA is included (Asad *et al*., 2003).

In 2002, Dharmacon was the first company to develop an algorithm for the rational design for highly potent siRNA gene silencing tool. The initial data for this discovery was published in Cell and Nature Biotechnology (Khvorova *et al*., 2003; Reynolds *et al*., 2004). Now there are several companies that have developed computer algorithms for analysis of a gene sequence based on a number of parameters and predict the most effective siRNA sequences for silencing that particular gene. There are several free software programs (applications) available on the internet for selecting the most effective region to be used for RNAi.

There are several interactive resources available on the internet free to use by any researchers. One would benefit by learning more about each software application and use the right tool to design silencing RNA fragment for a particular gene of interest. The web sites containing such RNAi related software applications are listed here.

- Silencing RNA Target Finder from Ambion **http://www.ambion.com/techlib/misc/siRNA_finder.html**

- RNAi Design Tool from Integrated DNA Technologies
  http://biotools.idtdna.com/rnai/
- Gene Specific siRNA Selector
  http://hydra1.wistar.upenn.edu/Projects/siRNA/siRNAindex.htm
- RNAi Designer from Invitrogen
  https://rnaidesigner.invitrogen.com/sirna/
- siRNA Selection Program, Whitehead Institute for Biomedical Research.
  http://jura.wi.mit.edu/siRNAext/register.php
- Summary of siRNA Design Rules and Tools
  http://www.protocolonline.org/prot/Molecular_Biology/RNA/RNA_Interference/siRNA_Design_Rules
- General Resources on RNAi in Ambion
  http://www.ambion.com/techlib/resources/RNAi/

RNAi AS A POTENT TOOL

The basic use of RNAi is to produce virus resistant crops. This natural process is interesting because it provides limits the viral infection (Voinnet and Baulcomb, 1997). Initially its importance was not recognized due to a misconception that it is limited to petunia with few other species (Cogoni and Macino, 1999). Nowadays it's the hot topic of molecular biology. RNAi is a favorable tool to knock down or silence a gene expression because it can target multiple gene family member by same RNAi inducing transgene. Another advantage of RNAi is related to its dominant aspect. It can knock down genes in polyploidy genome which contain four or more orthologs. Recent methods for RNAi silencing in plants are based upon an inverted repeat mRNA expression (Waterhouse *et al*., 1998). The best parts of these methods are that they are useful in both stable transformation and transient transfection. Few studies suggested an approach to improve the efficiency of silencing by the insertion of an intron between the inverted flanking target sequences (Smith and Gross, 2000). These RNA silencing strategies are suitable for crop improvement by reduce infection of RNA or DNA virus. Many plant viruse have ssRNA genome, which can deliver gene in PTGS system by inoculation of viral vector transcripts. In the case of DNA virus like geminivirus it becomes easier because it need only the viral DNA. Except this, cDNA of viral genome can be introduced in plant cell with the binary vector. There are several traditional gene knock-out methods which require transformation and tissue culture but PTGS is rapid and more effective.

RNA gene silencing is the mechanism which is conserved across the kingdoms. Interesting feature of geminiviruses is that they induce gene silencing without having dsRNA phase in their replication. They activate host PTGS system by the siRNAs which are specific for viruses. Geminiviruses encode AC2 and AC4 PTGS suppressor proteins that indicate the different evolution of this virus in respect to interaction with the host. This has been clear with the several studies that the geminiviruses and their statellite DNA are very useful for the gene silencing in various plants and also in post-genomic studies. Though a lot of studies have been done on RNAi mechanism related to geminiviruses but still many questions are there to be answered. As geminiviruse does not have any dsRNA structure in their replication cycle then how do they induce RNA silencing. There are some unproved theories which supports it, such as presence of overlapping transcripts and the presence of several mRNAs but no authenticated proof. How PTGS is controlled by suppressor proteins. Is there any inter-action of these suppressor proteins with miRNA pathway? The answers of these questions can help us to understand PTGS more precisely. As the new geminiviruses and their satellite DNAs are emerging rapidly, they can be devastating for the crops. To improve food production it must be controlled. For this we need to identify the common region of viral sequences to target a broad range of viruses and their PTGS suppressor system. RNAi or PTGS can be utilize as a potential tool against emerging geminiviruses to protect the crops. In respect to the present researches on RNAi can be considered as revolution in plant biotechnology and an effective tool in functional genomics. It's a broad application of biotechnology from molecular biology to gene therapy. Virus induced gene silencing has an enormous power to down regulate the endogenous genes that are virus-specific.

CONCLUSION

Bhendi yellow vein mosaic disease is the worst virus considered in the world which causes 50-90% crop losses. There are approximately 60 forms of viruses which affect crops. Current agricultural technology needs more and more molecular tools to reduce current crop loss and feed extra mouths, which according to a recent estimate by the FAO (Food and Agriculture Organization) will increase by two billion over the next 30 years. RNAi is the favorable tool to fight against this emerging threat of geminiviruses. RNAi is the pathway for silencing of whole gene families or unrelated genes through targeting of specific gene sequences or by using a single RNAi construct which contains several target sequencing. If judiciously used, this technology may go a long way to narrow the gap through production of disease, insect and virus resistant, nutritionally rich and toxic-free crops. Since this technology offers a great potential in understanding gene functions and utilize them to improve crop quality and production. Although the basic concept of the application of transgene-based RNAi to the genetic improvement of crop plants has been established, further feasibility studies are needed for its wider application.

REFERENCES

Abhary, M.K., Anfoka, G.H., Nakhla, M.K. and Maxwell, D.P. (2006) Post-transcriptional gene silencing in controlling viruses of the tomato yellow leaf curl virus complex. Arch. Virol., 151: 2349-2363.

Adjata, K.D., Muller, E., Aziadekey, M., Gumedzoe Y.M.D. & Peterschmitt, M. (2008) Incidence of cassava viral diseases and first identification of East African cassava mosaic virus and Indian cassava mosaic virus by PCR in Cassava (*Manihot esculenta* Crantz) fields in Togo. Am. J. Plant Physiol., **3:** 73-80.

Ahlquist, P. (2002) RNA-dependent RNA polymerases, viruses and RNA silencing. Science, 296: 1270-1273.

Ahmed, Z. & Patil, M. S. (2004) Incidence of yellow vein mosaic virus on different okra cultivars in Karnataka. J. Agric. Sci., 17(3): 615-616.

Anonymous (2007) Area and production of horticulture crops during 2005-06 to 2007-08. Ministry of Agriculture, India.(www.dacnet.nic.in/eands/At_Glance2008/ hpcrops).

Antignus, Y., Vunsh, R., Lachman, O., Pearlsman, M., Maslenin, L., Hananya, U. & Rosner, A. (2004) Truncated rep gene originated from Tomato yellow leaf curl virus-Israel (Mild) confers strain-specific resistance in transgenic tomato. Ann. Applied Biol., 144: 39-44.

Arguello-Astorga, G.R., Guevara-Gonzalez, R.G., Herrera-Estrella, L.R. & Rivera-Bustamante, R.F. (1994) Geminivirus replication has a group specific organisation of iterative elements: A model for replication. Virology, 203: 90-100.

Asad, S., Haris, W.A., Bashir, A., Zafar, Y., Malik, K.A. Malik, N.N. and Lichtenstein, C.P. (2003) Transgenic tobacco expressing geminiviral RNAs are resistant to the serious viral pathogen causing cotton leaf curl disease. Arch. Virol., 148: 2341-2352.

Bass, B.L. (2000) Double-stranded RNA as a template for gene silencing. Cell, 101: 235-238.

Baulcombe, D. (2004) RNA silencing in plants. Nature, 431**:** 356-363.

Berrie, L.C., Rybicki, E.P., Rey, M.E.C. (2001) Complete nucleotide sequence and host range of South African cassava mosaic virus: further evidence for recombination amongst begomoviruses. J. Gen. Virol., 82: 53-58.

Briddon, R.W., Watts, J., Markham, P.G. and Stanley, J. (1989) The coat protein of beet curly top virus is essential for infectivity. Virology, 172: 628-633.

Briddon, R.W., Mansoor, S., Bedford, I.D., Pinner, M.S. and Markham, P.G. (2000) Clones of cotton leaf curl geminivirus induce symptoms atypical of cotton leaf curl disease. Virus Genes, 20: 19-26.

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

Brunt, A.A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J. and Watson, L. (1996) Viruses of Plants-Description and lists from the VIDE Database. CAB International (Wallingford, UK). pp. 233-234.

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. J. Gen. Virol., 87**:** 3053-3065.

Capoor, S.P. & Varma, P. M. (1950) Yellow vein mosaic of *H. esculentus*. L. Indian J. Agric. Sci., 20: 217-230.

Carrillo-Tripp, J., Lozoya-Gloria, E. and Rivera-Bustamante, R.F. (2007) Symptom remission and specific resistance of pepper plants after infection by Pepper golden mosaic virus. Phytopathology, 97: 51-59.

Charrier, A. (1984) Genetic resources of the genus *Abelmoschus*. International Board for Plant Genetic Resources, Rome, pp. 21-41.

Chaudhary, D.R., Vidyasagar & Jagmohan, K. (1995) A note on the occurrence of yellow vein mosaic in intervarietal crosses of okra. Himachal Pradesh. J. Agric. Res., 21**:** 90-92.

Chauhan, D.V.S. (1972) Vegetable Production in India, 3rd ed., Ram Prasad and Sons (Agra).

Chellaiah, S. & Murugesan, S. (1976) Estimation of loss due to yellow vein mosaic disease in Bhendi. Madras Agric.J., 63: 169-170.

Chellappan, P., Vanitharani, R. and Fauquet, C.M. (2004) Short Interfering RNA Accumulation Correlates with Host Recovery in DNA Virus-Infected Hosts, and Gene Silencing Targets Specific Viral Sequences. J. Virol., 78**:** 7465-7477.

Cogoni, C. & Macino, G. (1999) Homology dependent gene silencing in plants and fungi: A number of variations on the same theme. Curr. Opin. Microbiol., 2**:** 657-662.

Covey, S. N., Al-Kaff, N. S., Langara, A. & Turner, D. S. (1997) Plants combat infection by gene silencing. **Nature, 385:** 781-782.

Day, A.G., Bejarano, E.R., Buck, K.W., Burrell, M. & Lichtenstein, C. P. (1991) Expression of an antisense viral gene in transgenic tobacco confers resistance to the DNA virus tomato golden mosaic virus. **Proc. Natl Acad. Sci. USA., 88:** 6721-6725.

Dry, I.B., Rigden, J.E., Krake, L.R., Mullineaux, P.M. & Rezaian, M.A. (1993) Nucleotide sequence and genome organization of tomato leaf curl geminivirus. J. Gen. Virol., 74**:** 147-151.

Elmer, J.S., Brand, L., Sunter, G., Gardiner, W.E., Bisaro, D.M. & Rogers, S.G. (1988) Genetic analysis of tomato golden mosaic virus II. The product of the AL1 coding sequence is required for replication. Nucl. Acid Res., 16: 7043-7060.

FAOSTAT. 2008. (http://www.fao.org)

Fauquet, C.M. & Mayo, M. A. (2001) The 7th ICTV report. Arch. Virol., 146: 189-194.

Finnegan, E.J., Margis, R. & Waterhouse, P.M. (2003) Posttranscriptional gene silencing is not compromised in the *Arabidopsis* CARPEL FACTORY (DICER-LIKE1) mutant ◆ a homolog of DICER-1 from Drosophila. Curr. Biol., 13**:** 236-240.

Gaur, R.K. & Rathore, M.S. (2009) RNAi mediated gene silencing against begomovirus in India. Project submitted to Department of Biotechnology, Govt. of India.

Grubben, G.S.H. (1977) Tropical agriculture and their genetic resources. FAO, Rome, p.120.

Hamilton, A. & Baulcombe, D.C. (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. Science, 286: 950-952.

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

Harrison, B.D. and Robinson, D.J. (1999) Natural genomic and antigenic variation in whitefly- transmitted Geminiviruses (Begomoviruses). Annu. Rev. Phytopathol., 37**:** 369-398.

Harrison, B.D., Muniyappa, V., Swanson, M.M., Roberts I.M. and Robinson, D.J. (1991) Recognition and differentiation of seven whitefly transmitted geminiviruses from India and their relationships to African cassava mosaic and Thailand mungbean yellow mosaic viruses. Annl. Appl. Biol., 118: 299-308.

Jeske, H., Lutgemeier, M., Preiss, W. (2001) DNA forms indicate rolling circle and recombination-dependent replication of Abutilon mosaic virus. EMBO.J., 20: 6158-6167.

Jha, A. & Mishra, J.N. (1955) Yellow vein mosaic of bhendi in Bihar. In: Proc. Bihar Acad. Agric. Sci., 4: 129-130.

Jose, J. & Usha, R. (2000) Extraction of geminiviral DNA from a highly mucilaginous plant (*Abelmoschus esculentus*). Plant Mol. Biol. Reptr., 18: 349-355.

Jose, J. & Usha, R. (2003) Bhendi yellow vein mosaic disease in India is caused by association of a DNA beta statellite with a begomovirus. Virology, 305: 310-317.

Khan, M. A. & Mukhopadhyay, S. (1985) Studies on the seasonal spread of yellow vein mosaic virus disease of okra. Indian Phytopath., **38:** 688-691.

Kheyr-Pour, A., Bendahmane, M., Matzeit, V., Accoto, G.P.M., Crespi, S. & Gronenborn, B. (1991) Tomato yellow leaf curl virus from Sardinia is a whitefly-transmitted monopartite geminivirus. Nucleic Acids Res., 19**:** 6763-6769.

Khvorova, A., Reynolds, A. & Jayasena, S.D. (2003) Functional siRNAs and rniRNAs exhibit strand bias. Cell, 115: 209-216.

Kulakarni, G. S. (1924) Mosaic and other related diseases of crops in the Bombay presidency. Poona Agricultural College Magazine, 16: 6-12.

Lana, A.F. (1976) Mosaic virus and leaf curl diseases of Okra in Nigeria. PANS (Pest Articles and News Summaries), **22:** 474-478.

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. J. Gen. Virol., 88: 3458–3468.

Lucioli, A., Noris, E., Brunetti, A., Tavazza, R. and Ruzza, V. (2003) Tomato yellow leaf curl Sardinia virus rep-derived resistance to homologous and heterologous geminiviruses occurs by different mechanisms and is overcome if virus-mediated transgene silencing is activated. J. Virol., 77: 6785-6798.

Martin, F. W. 1982. A second edible okra species and its hybrids with common okra. **Ann. Bot., 50:** 277-283.

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

Napoli, C., Lemiex, C. & Jorgensen, R. (1990) Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in Trans. Plant Cell, 2: 279-289.

Nath, P. & Saikia, A.K. (1995) Influence of sowing time on yellow vein mosaic virus on Ahmed, Z. and Patil, M. S., 2004, Incidence of yellow vein mosaic virus on different okra cultivars in Karnataka. J. Agric. Sci., 17(3): 615-616.

Navot, N., Pichersky, E., Zeidan, M., Zamir, D. & Czosnek, H. (1991) Tomato yellow leaf curl virus: A whitefly-transmitted geminivirus with a single genomic component. Virology, 185: 151-161.

Noueiry, A. O., Luces, W. J. & Gillbertson, R. L. (1994) Two proteins of a plant DNA virus co-ordinate nuclear and plasmodesmal transport. **Cell, 76:** 925-932.

Padidam, M., Sawyer, S., Fauquet, C.M. (1999) Possible emergence of new geminiviruses by frequent recombination. Virology, 265: 218-225.

Pinner, M.S., Medina, V., Plaskitt, K.A. & Markham, P. G. (1993) Viral inclusions in monocotyledons infected by maize streak and related geminiviruses. Plant pathol., 42: 75-87.

Pun, K.B. & Sabitha Doraiswamy (1999) Effect of age of okra plants on susceptibility to Okra yellow vein mosaic virus. Indian J. Virol., 15: 57-58.

Usha, R. (1980) In: Characterization, diagnosis & management of plant viruses, ed. by G.P. Rao, P.L. Kumar, R.L. Holguın-Pena (Studium Press, Houston,), pp. 387-392.

Reynolds, A., Leake, D., Boese, Q., Scaringe, S., Marshall, W. S. and Khvorova, A. (2004) Rational siRNA design for RNA interference. Nature Biotechnology, 22**:** 326-330.

Rojas, M.R., Noueiry, A.O., Lucas, W.J. & R. Gillbertson, L. (1998) Bean dwarf mosaic geminivirus movement proteins recognize DNA in a form and size specific manner. Cell, 95: 105-113.

Sanjaya, V.V.S., Prasad, V., Kirthi, N., Maiya, S.P., Savithri, H.S. and Sita, G.L. (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.

Sastry, K.S.M. & Singh, S.J. (1973) Restriction of yellow vein mosaic spread in okra through the control of vector, *Bemisia tabaci*. Indian J. Mycol. Pl. Pathol., 3**:** 76-80.

Saunders, K., Bedford, I.D., Briddon, R.W., Markham, P.G., Wong, S.M. and Stanley, J. (2000) A unique virus complex causes Ageratum yellow vein disease. Proc. Nat. Acad. Sci., 97**:** 6890-6895.

Schnippenkoetter, W.H., Martin, D.P., Willment, J.A., E. Rybicki, P. ((2001) Complete nucleotide sequence and host range of South African cassava mosaic virus: further evidence for recombination amongst begomoviruses. J. Gen. Virol., 82: 3081-3090.

Singh, S. J. & Dutta, O. P. (1986) Enation leaf curl virus of okra-a new virus disease. Indian J. Virol., 2(1): 114-117.

Sinha, S.N. and Chakrabarthi, A.K. (1978) Effect of yellow vein mosaic virus infection on okra seed production. Seed Res., 6: 67-70.

Smith, D.L. & Gross, K.C. (2000) A family of at least seven beta-galactosidase genes is expressed during tomato fruit development. Plant Physiol., 123**:** 1173-1183.

Tan, P.H.N., Wong, S.M., Wu, M., Bedford, I.D., Saunders, K. and Stanley, J. (1995) Genome organization of Ageratum yellow vein virus, a monopartite whitefly-transmitted geminivirus isolated from a common weed. J. Gen. Virol., 76: 2915-2922.

Thompson, C. H. & Kelly, C.W. (1957) Vegetable crops, Mcgraw Hill Book, Co. Inc., USA.

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

Voinnet, O. & Baulcomb, D.C. (1997) Systemic silencing in gene signalling. Nature, 389: 553-553.

Waterhouse, P.M., Graham, M.W. & Wang, M.B. (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, Z., Johansen, L.K., Gustafson, A.M., Kasschau, K.D. & Lellis, A.D. (2004) Genetic and functional diversification of small RNA pathways in plants. PLoS Biol., 2: E104-E104.

Zhou, Y.C., Nonssourou, M., Kon, M. R. Rajas, H. Jiang, Chen, L.F., Gamby, K., Foster, R., Gilbertson, R. L. (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. Arch. Virol., 153: 693-706.