Advances in Biotechnology

Chapter 4

RNAi based strategies for enhancing plant resistance to virus infection

*Veena Sinha[1]; Abhishek Anand[1]; Sunil K Mukherjee[2]; Neeti Sanan-Mishra[1]**

[1]*Plant RNAi Biology Group, International Center for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi-110067, India*

[2]*North Eastern Region-Biotechnology Programme Management Cell, New Delhi*

***Correspondence to: Neeti Sanan-Mishra,** Plant RNAi Biology Group, International Center for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi-110067, India. Tel: +91-11-2674 1358/61 (Ext. 280), Fax: +91-11-2674 2316; Email: neeti@icgeb.res.in*

Abstract

Viruses are the most potent parasitic entities that are detrimental to all animal and plant groups with no exceptions known so far. The viral genome though small is sufficient for sustaining the infection and its propagation inside the host organism. The sessile inhabitants of the plant kingdom have developed their strategies to counter this threat too with variable degree of success. The most important and significant of these strategies involves the RNA silencing mechanism. The viruses have evolved successfully in parasitizing and evading this plant defense strategy, by their ability to encode various "suppressor" molecules, which are able to target different components of the silencing pathway in plants. Besides suppression activity, these proteins also perform functions essential for virus multiplication and pathogenicity. In this chapter we briefly discuss about the plant defense strategies with the help of RNAi mediated processes with special focus on Virus Induced Gene Silencing (VIGS) and the viral suppressors as countermeasures to combat this strategy, while describing the probable mechanisms of suppressor action and the variations that exist in their mode of action. We have also tried to elucidate certain assays that are commonly used to detect and quantify the activity and strength of

these suppressor proteins. Then, we describe the specific applications of the RNAi based strategies used to counter virus attack.

Abbreviations: AGO: Argonaute; amiRNA: Artificial microRNA; CaLCV: Cabbage Leaf Curl Virus; Chs: chalconesynthetase gene; CMV: Cucumber Mosaic Virus; CP: Coat Protein; CP-MR: Coat Protein Mediated Resistance; DCL: DICER-Like; PTGS: Post-Transcriptional Gene Silencing; PVX: Potato Virus X; PVY: Potato Virus Y; ra-siRNA: Repeat associated siRNA; RdRP: RNA dependent RNA polymerase; REP: Replication-associated protein; RISC: RNA Induced Silencing Complex; RSS: RNA Silencing Suppressor; siRNA: Small interfering RNA; SVISS: Satellite-virus-induced silencing system; dsRNA: Double stranded RNA; GUS: β-glucoronidase; Hc-Pro: potyvirus encoded helper component proteinase; miRNA: microRNA; MYMIV: Mungbean Yellow Mosaic India Virus; nat-siRNA: Natural antisense siRNA; Pds: phytoenedesaturase; ta-siRNA: Trans acting siRNA; TE: Tobacco Etch Virus; TGMV: Tomato Golden Mosaic Virus; TGS: Transcriptional Gene Silencing; TMoV: Tomato Mottle Virus; TMV: Tobacco Mosaic Virus; ToLCV: Tomato Leaf Curl Virus; TRV: Tobacco Rattle Virus; TYLCV: Tomato Yellow Leaf Curl Sardinia Virus; vi-siRNA: Viral siRNA; VIGS: Virus Induced Gene Silencing

Keywords: RNA Silencing; Viral suppressor; Mechanism; double stranded RNA; VIGS

Mishra NS

1. Introduction

Viruses are recognized as the primary cause of nearly half of the infectious diseases in plants. Transmitted by insects such as leafhoppers, treehoppers, whiteflies etc the viruses are capable of infecting almost all types of plants. Infected plants may show a range of symptoms depending on the disease but often there is yellowing of leaf (streaking, vein clearing or mosaic), curling of leaf and other growth distortions like plant stunting, abnormalities in flower or fruit formation etc. The infecting viruses can damage up to 70% to 100% of yield [1] and rough predictions indicate that the total worldwide damage due to plant viruses may be to the tune of US$ 60 billion per year. The accumulation of vast amount of data from across the globe has revealed the devastating potential of the different viruses and have established their identity as notorious plant pathogens.

Plants have developed strategies to counter this threat with variable degree of success. The strategy based on the RNA silencing pathway being the most important and significant. It is a method of sequence-dependent gene regulation involving suppression of transcription, transcript degradation or translation inhibition [2,3]. The phenomena of RNA silencing was first observed during loss in petunia flower pigmentation while over expressing the chalcone *synthetase (chs)* gene [4]. An important observation on RNA silencing was made during experiments with pathogen-derived resistance when it was shown that virus resistance correlated with reduction of viral mRNA in the cytoplasm. Later three independent reports demonstrated that untranslatable viral RNA was sufficient to produce virus resistant plants and the expression of viral proteins was not required [5-7].

These findings launched the search for the “resistance factors” and in the year 1999, it was explicitly proved that plants accumulated small double stranded RNA (dsRNA) molecules whose sequence was identical to the silenced transgene [8]. Similarly sequence-specific small

RNAs were observed in *Potato Virus X* (PVX) infected plants, suggesting a role of these molecules in antiviral defense mechanism. This triggered the discovery of the components and pathways of the silencing machinery [8]. Since then, it has evolved as a gene silencing tool with great potential for virus resistance. The silencing mechanism is involved in adaptive defense response, gene regulation and chromatin maintenance [9-11]. Though the virus can act as both inducers and targets of gene silencing, they have evolved successfully in parasitizing and evading plant defence strategies, by their ability to encode various "suppressor" molecules which are able to repress different components of the silencing pathways. An understanding of both components is necessary for developing effective antiviral strategies for enhancing plant resistance.

2. RNA silencing in plant antiviral defense

RNA silencing or RNA interference is the natural strategy of switching off gene expression during fundamental processes like development, genome maintenance and defence against foreign molecules e.g. viruses. With the rapid advancement in science, a lot of information has emerged regarding the mechanisms and machineries of RNA silencing [12]. This is being exploited as a new tool for developing antiviral products, which have large applications in field of medicine, agriculture and basic biology [13]. In the medical sector, several studies have demonstrated efficient *in-vivo* delivery of siRNAs and therapeutic benefit in mice or bovine models. Presently several companies are engaged in developing RNA silencing based drugs for clinical use [14]. In the agricultural sector studies have been performed on a number of plants to improve nutritional content, increase yields and remove undesirable metabolites [15]. The emphasis is on deciphering gene functions and identifying pathways that can be directed to protect plants from environmental perturbations and pathogen attack. The potential of RNAi has been recently demonstrated in developing effective resistance against many coleopteran and lepidopteran insect-pests of crops [16] and in managing plant-parasitic nematodes [17].

The RNAi mechanism exhibits an array of diversity in different components for its mode of action but the basic mechanism involves the cleavage of a stem-loop like or a perfect dsRNA structure into small RNA molecules of about 21-24 nt length. This inducer dsRNA can be endogenous like annealed overlapping transcript of opposite polarity, triggered by transcription of tandem or inverted repeat sequences or else exogenous, like RNA virus replication intermediates [18-23]. The dsRNAs are diced into RNA duplex of 20-24 nts with the characteristic 2 nt 3' overhangs by DICER, which is a key component of the microprocessor complex [8,19,24,25]. The small RNAs associate with a set of proteins to form RNA Induced Silencing Complex (RISC), which directs the silencing pathway. The small RNAs include the miRNAs (microRNAs) and the siRNAs (small interfering RNAs) with their various sub-types, viz., ta-siRNA (trans acting siRNAs), ra-siRNA (repeat associated siRNAs), vi-siRNA (viral siRNAs), nat-siRNA (natural antisense siRNAs) etc. Several excellent reviews are available

detailing the nature, classification, biogenesis and function of these small RNAs [3,26-29].

The key protein molecules involved in the process of small RNA biogenesis and function are DICER (DCL), RNA DEPENDENT RNA POLYMERASE (RdRP) and ARGONAUTE (AGO). The silencing mainly occurs at two different stages in cells and hence is categorized accordingly. The cytoplasmic RNA silencing also known as Post-Transcriptional Gene Silencing (PTGS), targets mRNA for degradation or translation repression with the help of 21-22 nt species of RNA molecules generated from inducing dsRNA [2]. This involves various small RNA classes (miRNA, siRNA, trans-acting siRNA) and DCLs. Subsequently the diced small RNAs are loaded into the RISC effector complex to guide specific localized silencing [18-22,30]. In several cases, the primary siRNAs prime the RdRP6 mediated synthesis of secondary dsRNA for generation of secondary siRNA or transitive siRNAs. This results in the amplification of siRNAs and the spread of silencing beyond the site of its initiation to bring about systemic silencing [2].

The other silencing pathway operates at the nuclear level and is called Transcriptional Gene Silencing (TGS). This pathway is directed by the 24 nt siRNAs and miRNAs and it involves heterochromatin silencing by cytosine methyation of DNA and post-transcriptional modifications of histone proteins (e.g. H3- methylation at lysine – 9). The siRNA involved in this case are generated by DCL-3 with the help of AGO-4 and RdRP-2 [2,3]. This pathway is considered significant in preventing rearrangement in centromeric and telomeric repeats by suppressing transposons and other invasive DNAs and thus maintaining genetic integrity [2, 3].

2.1 Virus induced gene silencing

The observations that viruses act as inducer as well as target of RNAi machinery lead to the theory that the silencing mechanism is primarily a defense system in plants [31,32]. The invading viral RNAs can precondition this response, even though the natural response is adaptive and requires recognition of ‘foreign’ nucleotides for initiation [33-35]. The small RNAs triggered in response to one mild virus also serve as “molecular memory” to cross-protect the infected plant against virulent infection by another related virus carrying sequences homologous to the first virus [33]. This cross protection phenomenon was reported by the plant virologists as early as 1920, though the mechanisms were worked out much later.

In fact, the term Virus Induced Gene Silencing (VIGS) was first used to describe the phenomenon of recovery from virus infection [36]. Soon after virologists observed that over expression of certain genes using viral vectors led to the degradation of the desired mRNA resulting in genotypes resembling a nearly knockout mutation of the corresponding gene. This was explained to be due to RNA silencing and subsequently the phenomenon of VIGS was exclusively used to describe the ability of recombinant viruses to knock down expression of en-

dogenous genes [37,38]. It was speculated that majority of plant viruses replicate via a dsRNA intermediate which may serve as the principal inducer of the siRNA/RNase system resulting in VIGS. The secondary structure or convergent transcription of viral RNAs can also serve as a potent trigger of RNA silencing and the reaction is further amplified by host encoded RdRPs [39,40]. Thus RNA silencing stands as a very important as well as efficient tool for plants to maintain their defence strategy, especially against viruses.

VIGS as a tool is gaining immense popularity in the field of functional genomics as it is a simple method when compared to agroinfiltration or biolistic gene guns. The method does not involve stable transformation and the results can be obtained rapidly within a period of 2-3 days only. Furthermore, it is easy to use and provides a high throughput characterization of phenotypes that might be lethal in stable lines. It also has the advantage of being very specific to the target and has been successfully used for rapid silencing of one or more genes in a large number of species. However, it may have limitations on availability of infectious clones, its host range, regions of silencing and size restrictions on the inserts. Furthermore, the virus in the VIGS vector needs to be disarmed to avoid any symptom development due to infection [41].

VIGS is being used as a tool that employs the RNA mediated antiviral defence mechanism to produce gene knockouts. A number of VIGS vectors have also been constructed by cloning the gene to be silenced in a minimal portion of the viral DNA (or cDNA in case of RNA virus) that could efficiently replicate and cause infection [38]. This vector is then introduced mechanically into the system and whenever a dsRNA structure or a secondary RNA structure is formed during the course of infection, silencing process is efficiently initiated against the infecting virus. In the process the host silencing response also silences the target-gene(s) at the post-transcriptional levels, in a sequence-specific manner [42]. The first VIGS vector was developed using *Tobacco Mosaic Virus* (TMV) which has an RNA genome. Transcripts of recombinant TMV carrying a sequence encoding *phytoene desaturase* (*pds*) were produced *in vitro* and inoculated to *Nicotiana benthamiana* plants leading to successful silencing of *pds*. The leaves of these plants exhibited characteristic white patches due to photo-bleaching as PDS enzyme is envolved in biosynthesis of carotenoids that protect the chlorophyll from photo-bleaching [43].

Tobacco Rattle Virus(TRV) based VIGS vectors are the most widely used. These are usually cloned between the T-DNA borders and introduced in plantsby agroinfiltration (44-48). TRV-based VIGS vectors have been used to silence genes in a number of Solanaceous plant species [47,49,50]. PVXbased VIGS vector have also been used, however they have more limited host range as only three families of plants are susceptible to this virus(46). The geminiviruses like bipartite *Cabbage Leaf Curl Geminivirus* (CaLCV), *Mungbean Yellow Mosaic India Virus* (MYMIV), *Tomato Golden Mosaic Virus* (TGMV), etc have emerged as very

promising DNA virus based VIGS vectors, as they can be delivered by direct plasmid DNA infection. The *Tomato Leaf Curl Virus* circular replicon based VIGS vector was shown to silence the *pds* and*pcna*genes of tomato in a long-lasting manner [51]. A list of commonlyused VIGS vectors is provided in Table 1.

The Satellite-virus-induced silencing system, SVISS, was also demonstrated as an efficient gene silencing in plants.It employs the dual-component of a Satellite-virus-based vectorand ahelper virus. The first SVISS was based on a Satellite virus which uses the TMV strain U2 as a helper [53]. In other studies,modified satellite DNA were used for silencing genes alongwith *Tomato Yellow Leaf Curl China Virus*in *N. Benthamiana* [65], *African Cassava Mosaic Virus*in cassava [67], *Pea Early Browning Virus* in pea [56] and *Bean Pod Mottle Virus* in soybean [58]. This method has the advantage ofbeingeasily clonable (small genome size), highly stable and showing attenuated symptoms of virus infection.

3. Suppressor of RNAi

Viruses have evolveda defense measure for evading the RNA silencing mechanisms. They encode for protein molecules known as 'suppressors' [68,69] which interfere at different stages of RNA silencing pathway thus helping in efficient infection and replication of virus in the host cell and spreading the infection systemically. These suppressors molecules are usually ordinary viral proteins e.g., coat protein, movement proteinor proteases that carry the suppressor activity as their secondary function. It has been suggested that the suppressor activity is casually coupled with transcription factor activity [70]. As a result there is extensive assortment in the RNA Silencing Suppressors (RSS) documented from the distinct viruses. A number of suppressors discovered so far in various systems have been listed in Table 2.

3.1. History of RNA silencing suppression

The vital role played by specific virus encoded proteins in augmenting virulence provided the first indication on the presence of RSS. In the classical study it was shown thatPVX by itself, causes mild symptoms but the symptoms show a vigorous enhancement during co-infection with the *Potato Virus Y* (PVY) and *Tobacco Etch Virus* (TEV) [31]. Subsequently several other reports showed that co-infection with combination of viruses caused increased symptom severity compared to each of the viruses alone.This phenomenon was denoted as synergism [71] and it is now understood that the enhanced synergism ismainly due to weakening of the host defense by RSS targeting the silencing pathway at multiple points [31,72].

In 1998 several independent reports showed the involvement of a *potyvirus* encoded helper component proteinase (Hc-Pro) in enhancement of replication of many unrelated viruses. In one such report it was shown that Hc-Pro suppressed the PTGS of β-glucoronidase (GUS) reporter transcript on a highly expressed locus [92]. In another study, GUS silenced

*Nicotiana tabacum*plants were crossed with four independent transgenic plants expressing TEV-P1/HC-Pro and it was observed that silencing was reversed in the resulting offsprings [87]. These observations led to identification of P1/Hc-Pro as the first RSS.

In the same year, Brigneti and co-workers showed that PTGS of a green fluorescent protein (GFP) in *Nicotiana benthamiana* plants transgene is suppressed after infection with *Cucumber Mosaic Virus* (CMV). In an interesting experiment they expressed HC-Pro of PVY and 2b protein of CMV-encoded proteins in a PVX vector and demonstrated that both can act asRSS. They also demonstrated that HC-Pro acts by blocking the maintenance of PTGS in tissues containing established silencing, whereas the 2b protein prevents initiation of gene silencing in the newly emerging tissues [81]. Since then several RSS from plant, insect (like B2 protein of *Flock House Virus*) and animal (like NS1 encoded by *Influenza Virus*) viruses have been identified [87,93,94]. It was also shown that the RSS can suppress silencing in both animal and plant cells, regardless of their host preference due to the conserved nature of the silencing phenomenon [95].These findings triggered the search for more RSS and since then, a number of viral encoded RSS have been discovered (Table 2). It emerges that the viruses employ RSS as a common strategy against one of the most potent induced defense system.

3.2. Identification of RNA silencing suppressors

The analysis of candidate viral proteins as potential RSS was enabled by the development of different screening systems, based on monitoring their role in reversing the RNA mediated silencing of reporter genes like GFP or GUS. The assays utilized different reporter constructs, such as partial or complete inverted repeats and this also provided an indication on the possible site of action of the RSS [96]. The most commonly used *in planta* assay is based on transgenic tobacco plants constitutively silenced for a reporter gene [97]. The RSS activity can beassayed by rummaging for localized reporter gene expression following transient expression of the virus encoded protein [98,99]. As a modification of this method the reporter gene and the putative RSS are co-infiltrated in wild type tobacco leaves followed by monitoring the reporter gene expression [86,100,101]. It is anticipated that in presence of RSS activity, the reporter gene expression will be retained to a high level or may even increase.

An alternative method involves generating two types of stable transgenics, one containing a silenced reporter gene and the other expressing the candidate viral RSS [87,92,102-104]. The two plants are crossed and the progeny is screened for reporter gene expression. However, this method is labour intensive and often over-expression of RSS in the plants affects seed formation and leads to developmental defects. Alternatively, the candidate RSS can be ectopically expressed from a heterologous viral vector, which is inoculated on to the silenced transgenic plants. PVX based vectors lack the ability to restore the reporter gene expression in such assays and thus serve as suitable vectors to test the viral genes for their RSS capability [81].

The affect of RSS on systemic movement of the silencing molecules can be assayed by grafting of a reporter gene silenced rootstock to a reporter gene expressing scion. The silencing signal systemically spreads from rootstock to scion to silence it reporter gene expression. The candidate RSS is introduced in the plant serving as the rootstock with the help of genetic crossing. In presence of RSS activity the spread of the silencing signal will be curtailed and there will be no effect on the reporter gene expression in the scion. This assay is not only time consuming but requires the production of transgenics as well as breeding experiments

3.3. Mechanism of RNA silencing suppression

The RSS encoded by different plant viruses appear to suppress the silencing based virus defense pathway in different points [68,86,100,105-107]. They primarily act on the common biogenesis or functional components of the pathway causing suppression of the siRNA-mediated [108-113] pathways, resulting in breakdown of the host anti-viral defense response [96]. The common sites of action include:

3.3.1. Binding double stranded RNA

In the silencing pathway the long dsRNA actsas a major inducer and small dsRNA serves as a major effector molecule. The binding of RSS masks the long dsRNA and thus protects it from the DCL action, thereby preventing its processing into siRNA [114]. The binding to small dsRNA like siRNA duplex prevents their sorting into the AGO containing RISC complex and renders them functionally inactive. Binding to small RNA duplex is a common strategy for many of the viral encoded RSS [114] encoded by phylogenetically and evolutionarily divergent viruses like tombusvirus P19, closterovirus P21, carmovirus CP, pecluvirus p15, hordeivirus QB, potyvirus HC-Pro, CMV-2b [115-120].

The RSS also interfere with miRNA biosynthesis in plants and inhibit the cleavage and translational repression of target genes by specific miRNA in the plant developmental pathway [101,102-104,121-124]. In plants, virus-induced disease symptoms often result in developmental abnormalities resembling perturbation of miRNA-mediated function. Several studies have now shown that transgenic expression of RSS can alter the accumulation and/or functioning of miRNAs leading to developmental abnormalities related to the action of miRNAs [125]. Tombus virus encoded P19, *Beet Yellow Virus* encoded P21 and Potyvirus P1/HC-Probind to duplex forms of miRNAs [103].

The crystal structure of RSS further indicates that themechanism of RNA binding also varies [126]. TAV2b recognizes siRNA (19nt) duplex by a pair of hook-like structures and adopts a R-helix homodimer structure to measure siRNA duplex in a length-preference mode, whereas P19 protein uses an extended S-sheet surface and asmall R-helix to form a caliper-like architecture for binding and measuring thecharacteristic length of siRNAs.Few RSS proteins

have the ability to bind both long and short dsRNA, like p14, FHV-B2 [120]. AC4 protein of ACMV is a unique RSS because of its ability to bind to single-stranded forms of miRNAs and siRNAs and thus interferes with both miRNA-mediated function and suppression of siRNA-mediated PTGS.

3.3.2. Binding to biogenesis components

Many RSS have been found to interact with and inhibit the activities of DICER/DCL and this directly affects the small RNA biogenesis. P38of *Turnip Crinkle Virus* suppresses DCL4 activities [127]. The P6 protein of CMV suppresses the host DRB4 activity which is required to facilitate the activity of DCL4 enzyme (128). Among the non-plant virus FHV-B2 interacts with the PAZ domain of DICER (120)and the Hepatitis C virus core proteinalso directly interacts with DICER to antagonize RNA silencing [126].

RDR6 is another important component mainly associated with sense gene mediated silencing and transitive siRNA biogenesis by generating dsRNA. MYMIV-AC2 has been found to be interacting with RDR6 to interfere with RNA silencing [129]. Potyvirus P1/HC-Pro also interferes with the HEN-1 mediated methylation of miRNA [103].

3.3.3. Interference with RISC

Suppression activity at RISC level is achieved by targeting the AGO protein. Polerovirus encoded P0 and CMV-2b suppresses RNA silencing by destabilizing the AGO1 [130,131]. Similar silencing mechanism has also been observed in case of MYMIV-AC2 [129]. P1/HC-Pro also inhibits miRNA-mediated cleavage of target mRNAs, but the exact mode of action of this protein in the silencing pathway is not known.

3.3.4. Interference with DNA methylation

Some RSS have the ability to reverse the small RNA mediated TGS. They can cause reversal of TGS by various mechanisms. It has been reported that the 2b protein of severe Shan-Dong (SD) isolate of *Cucumber mosaic virus*, suppresses RdDM by binding and sequestering siRNAs in a process involving AGO proteins in the nucleolus [132]. Another mechanism is displayed by the AC2 protein of Begomovirus and Curtovirus genera. They inactivate Adenosine Kinase, thereby reducing production of the methyl donor (SAM) and causing release of TGS [133,134]. The C1 protein of beta-satellite of *Tomato yellow leaf curl china virus* inactivates S-Adenosyl homocysteine Hydrolase, an enzyme required for synthesis of SAM and thus reduces the level of cytosine methylation of viral DNA [135]. The C2 protein of *Beet severe curly top virus* increases the life-span of SAMDC1 and thus suppresses DNA-methylation mediated gene-silencing in Arabidopsis [136]. The AC2 protein of *Indian cassava mosaic virus* up regulates RAV2, which acts as a transcriptional repressor, inhibiting transcription of

KYP, a histone methyl transferase.

4. Applications of RNA silencing to counter virus attack

The viral pathogens depend on the host's cellular machinery for reproduction; hence it is challenging to eliminate them without damaging the host plant. Therefore, most management strategies for diseases caused by plant viruses are directed at preventing infection. The RNA silencing based strategies to induce a highly specific antiviral state in plantshave been extensively employed to raise virus resistant transgenic plants, even before the exact mechanisms were comprehended. This is evident from the commercial cultivation of*Papaya Ringspot Virus* resistant 'SunUp' papaya and virus resistant potato varieties "NewLeaf Plus" and "NewLeaf Y". The understanding of the VIGS and RSS helped to further refine the techniques. The targeting of the RSS, which play an important role in viral pathogenecity, emerged as efficient antiviral strategy. Ever since the anti-viral properties of RNAi have been tested in many other crops.

4.1. Pathogen –derived resistance

Sanford and Johnston [137] developed a simple concept of parasite or pathogen-derived resistance to explain the observations that a plant infected with one virus shows resistance to infection by same or a closely related virus strain.This concept was utilized in the early attempts to engineer resistance through the introduction of viral genetic material into the plant genome [138,139].

An excellent example is provided by the pioneering work of Robert Beachy's group in 1986 by providing coat protein (CP)-mediated resistance to TMV [140]. It was demonstrated that the over-expression of viral CP gene in transgenic plants could protect these plants from the infectionby TMV and closely related tobamoviruses. It was shown that transgenically expressed CP interfered with the assembly of TMV particles due to hindrance with the inter-subunit interactions [141]. These results also indicated that plants could be genetically transformed for resistance to virus disease development and the trait of resistance could be stably transmitted to the next generation [142]. This phenomenon was referred as coat-protein-mediated resistance (CP-MR) and was found to be effective in a variety of host or virus combination. CP-MR thus became a choice for the researchers to develop transgenic plants against various viruses. The CP-MR was used against the*Rice Stripe Virus* by introducing its CPin japonica rice by protoplast electroporation followed by generation of transgenic plants (143). CP-MR was also reported for *Potato Virus Y* in tobacco [144]; *Tomato Yellow Leaf Curl Virus* (TYLCV) in tomato (145); *Pea Enation Mosaic Virus* in pea [146]; *Potato Mop-Top Virus* in potato [147] and *Cucumber Mosaic Virus* 1B in tobacco [148].

In viruses the CP plays an important role in ssDNA protection, movement and transmis-

sion of viruses. A CP-deficient ACMV-KE clone lost its systemic infection capacity in cassava plants and showed reduced functional interaction with its vector *Bemisia tabaci* [149]. The vector specificity determinant regions were also shown in the CP of *Abutilon mosaic virus* [150]. Expression of a mutated non-functional CP therefore appeared to be apotential strategy to impede the virus spread amongst its vectors. Later, it was shown that, mutant forms of TMV CP had stronger inter-subunit interactions and these were found to confer increased levels of CP-MR compared with wild-type CP.

The viral replication-associated protein (REP) also emerged as a strong candidate for pathogen-derived resistance. The genomes of plant single-stranded DNA viruses do not encode polymerases, buttheir replication requires interaction between the REP and host polymerases. The Rep protein by itself is not a determinant of disease or pathogenesis [151]. It was shown that over-expression of truncated *rep* gene (encoding for the N-terminal 210 amino acidsof REP) showed resistance against *Tomato Yellow Leaf Curl Sardinia Virus* up to 15 weeks post virus inoculation [152,153]. Similar observations were reported for the expression of the N-terminal region encompassing the DNA binding and oligomerization domain of *Tomato leaf curl New Delhi Virus*. This also accorded resistance to heterologous ACMV, *Pepper Huasteco Yellow Vein Virus*and *Potato Yellow Mosaic Virus* [154].

The virus also encodes movement proteins (MP) that are required for cell-to-cell and long-distance movement. Tobacco plants expressing *Tomato Mottle Virus* (TMoV) encoded movement proteins, BV1 or BC1, showed a significant delay in infection to TMoV and CaLCV infection [155,156]. Non-functional MPs may compete for Nuclear Shuttle Protein; required for long distance movement in begomoviruses interaction or oligomerization and this could explain the resistance previously observed in mutated MP expressing plants [157]. However the overexpression of these proteinsalso had deleterious effects on plant development [158]. Over the years, other full-length or truncated viral genes, like RdRp, proteinase, satellite RNA, defective interfering RNA, and noncoding regions, have been extensively used to engineer virus resistance.

4.2. Antisense RNA

Regulation of gene expression by antisense RNA (asRNA) was first discovered as a naturally-occurring phenomenon in bacteria [159], however its effectiveness in eukaryotic cells was demonstrated as early as 1984 [160]. The antisense technology is based on blocking the information flow from DNA via RNA to protein by the introduction of an RNA strand complementary to the sequence of the target mRNA. It was hypothesized that the antisense RNA base-pairedwith its target mRNA thereby forming dsRNA duplex causing the blockageof mRNA maturation and/or translation [161]. It was later shown that the dsRNA is recognised as a substrate by the RNA silencing machinery resulting in its rapid degradation into siRNAs

which in turn cleave the target mRNAs in a sequence dependent manner. The antisense technology has potential as therapy to treat many genetic and metabolic disorders, for identifying gene functions and in crop development.

The application of artificial antisense RNA was demonstrated in plants by down-regulating the *chs* gene which is responsible for flavonoid biosynthesis [162,163]. The chs-antisense RNA elicited increased fungal disease susceptibility in *Arabidopsis* plants [164]. This technology was successfully used in Flavr Savr to delay tomato ripenining and rotting by introducing the antisense RNA for *Polygalactourodase* gene to inhibit the synthesis of the enzyme [165,166]. The expression of antisense RNA against *Potato Leaf Roll Luteovirus* CP triggered virus resistance in the transgenic plants [167]. Day and coworkers [168] used antisense AL1 transcripts of TGMV to engineer geminivirus resistance in tobacco plants. Resistance was engineered against the *Cotton Leaf Curl Virus*by using anti-sense constructs of Rep, REn and TrAP genes [169]. The antisense technology was also effective in confirming resistance against infection by viruses like PVX [170], TMV [171], CMV [171] and TYLCV in *Nicotiana benthamiana* [172] and tomato [173].

4.3. Hairpin RNA and double stranded RNA

The direct application of RNA silencing in plants was initiated by theuse of intron loopedself complimentary hairpin RNA (hpRNA) constructs [174]. The hpRNA constructs contained, 100-800 bp long fragments of the target gene cloned in sense and antisense orientations, separated by an intron sequence. When transcribed *in planta* the primary transcripts folded into a hairpinstructure, which could be recognized and processed by DCLs into siRNAs [175]. The siRNAs then induced PTGS and repressed the target gene strongly. These were found to be more effective in silencing the target genes as compared to overexpression of antisense transcripts. The hpRNA have emerged as the reagents of choice for triggering specific RNAi against a variety of viruses in different plant species [176-178]. These constructs are delivered into the cells through agrobacterium or gene gun as plasmid or viral vectors where the get transcribed and processed into sRNAs. The hpRNA encoding constructs driven by a maize ubiquitin promoter bestowed immunity to transgenicbarley against *Barley Yellow Drawf Virus* [179]. Transgenic plants resistant to *Cassava Latent Virus*were produced by introducing a tandem repeat of its subgenomic DNA B [180]. HpRNA construct with 424bp conserved region of *Bean Golden Mosaic Virus* REP was used to generate virus resistant tobacco plants [181]. Recently it was reported that a spray application of a long noncoding dsRNA on barley plants was effective in targeting three fungal cytochrome P450 *lanosterol C-14α-demethylases*, required for biosynthesis of fungal ergosterol [182]. Similarly wheat plants pre-infected with BSMV containing antisense sequences against target genes of the fungus, *Fusariumculmorum* caused a reduction of corresponding transcript levels in the pathogen and reduced disease symptoms [183]. Similar efforts are ongoing to leverage the power of RNAi in engineering

effective, broad-spectrum and ecologically safe resistance against many viruses in different crops [184-193]. The major limitation of this technology lies in the generation of aberrant siRNAs, which result in silencing of the non-specific genes.

With the unveiling of the mechanism of RNA silencing it was clear that the dsRNA serves as the trigger for PTGS, so strategies based on introduction of dsRNA were also employed. The major bottleneck for the application of this technology lies in the proper designing and effective delivery of siRNAs molecules.Several online softwaresare now available to design the correct siRNAs [28]. The choice of delivery technique(s) is more or less governed by the preparation of the siRNA. Among the popular approaches for siRNA production are chemical synthesis, *in vitro* transcription, expression vectors and PCR expression cassettes. It is important to adapt the correct delivery strategies to facilitate better cellular accumulation of siRNAs andtheir releaseclose to the respective targets site.It is generally believed that the siRNAs are passively endocytosed [184] however,the in vivo transmission of naked siRNAs in-vivo is limitatedby inefficient cellular uptake, nucleolytic degradation and other problems like trapping in non-desirable cellular compartments. Hence direct application siRNA involves mixing these molecules with 'biocompatible' and 'genocompatible' formulations for appropriate delivery [185].

In plants, generally vectors are used to generate and deliver siRNAs to the target tissues instead of direct delivery. The use of viral vectors has been discussed in the section 2,1 under VIGS. The plasmid vectors can exist as episomes or integrate in the genome. The plasmids are used to express around 70-nt shRNAs or hpRNAs, which are transcribed into effective dsRNAs capable of silencing the target genes [186,187]. One of the excellent application is the use of specific dsRNA and siRNAs for HCPro region to block the replication and transmission of PVY through the potato plants [194].

The dsRNA also induces genomic methylation [195]. Methylation of the promoter sequence induces TGS, which unlike PTGS is stable and heritable. However if methylation occurs in the coding sequence, it has no apparent effect on the transcription of the locus, although silencing still occurs at the post-transcriptional level. The dsRNA carrying a sequence homologous to the promoter of the transgene can guide the methylation and TGS in plants. Methylation of a *Tomato Leaf Curl Virus* -derived transgene promoter and consequent transgene silencing have been observed on infection [196] strongly suggesting that virus-derived siRNAs are also generated for the transcribed and non-transcribed intergenic regions of the viral genome [197]. The dsRNA-guided methylation of geminivirus bidirectional promoters may down-regulate the transcription of viral genes, resulting in inefficient virus replication [198].

4.4. Artificial miRNA

Artificial microRNA (amiRNA) technology is based on designing miRNA or engineering miRNA artificially by mimicking the intact secondary structure of endogenous miRNA precursors to utilize thenatural silencing pathway to target desired transcripts [199,200]. It has been shown that altering several nucleotides within sense and antisense strands of miRNA has no bearing on its biogenesis and maturation, as long as secondary structure of its precursor remains unaltered. The amiRNA acts as a specific, powerful and robust tool that can be applied to study metabolic pathways, gene functions and for improving favourable traits. The amiRNA technology was first used for gene knock down in human cell lines [201] and later in Arabidopsis [202], where they were shown to effectively interfere with reporter gene expression. Subsequently, it was demonstrated that amiRNAs when expressed under constitutive or tissue-specific promoters can down-regulate a number of endogenous genes without affecting the expression of other unrelated genes [124,203].

The amiRNA sequences are designed according to the determinants of plant miRNA target selection, such that the 21 nt sequence specifically silences its intended target genes. They resemble the natural miRNAs in containing a Uracil residue at their 5' end; having an Adenine/ Uracil residue as their 10^{th} nucleotide and displaying 5' instability relative to their amiRNA* sequence [204,205]. The miRNA mediated gene regulation has also emerged as a second generation tool in the field of RNAi technology and having various applications in the field of agriculture, medicine and in the field of functional genomics studies [203, 206-211].

The amiRNA technology is being utilized to target the invading viral gene transcripts and the effective strategies employ targeting the viral encoded RSS transcripts [212]. The amiRNAs arising from ath-miR-159 backbone were effective in targeting viral sequences encoding RSS, P69 of *Turnip Yellow Mosaic Virus* and HC-Pro of *Turnip Mosaic Virus* [206]. Similarly, amiRNA sequences targeting 2b of *Cucumber Mosaic Virus* (CMV), a potent RSS, can confer effective resistance to CMV infection [208]. The amiRNA targeting overlapping regions of geminiviruses genes, AC1, AC2 and AC4 were used to generate transgenic tomato plants that could resist infection of ToLCV New Delhi variety [213,214]. These amiRNAs were also used for generating resistance against*Watermelon Silver Mottle Virus* in tobacco [215].

4.5. Artificial tasiRNA

Artificial tasiRNA technologyhas also been used to generate virus resistant plants. The strategy involves designing a binary vector incorporating the 5' and 3' binding sites of miR390 flanking the RSS sequence on each side, respectively. This vector when introduced in plants produces artificial tasiRNAs from the RSS encoding sequences. These tasiRNAs can inactivate the viral transcripts containing homologous sequences. The transgenics producing the artificial tasiRNAs were used to protect the plants against the invading ToLCVs [216]. Such

strategy could in principle be adopted to develop plants tolerant for other phytoviruses.

5. Figure

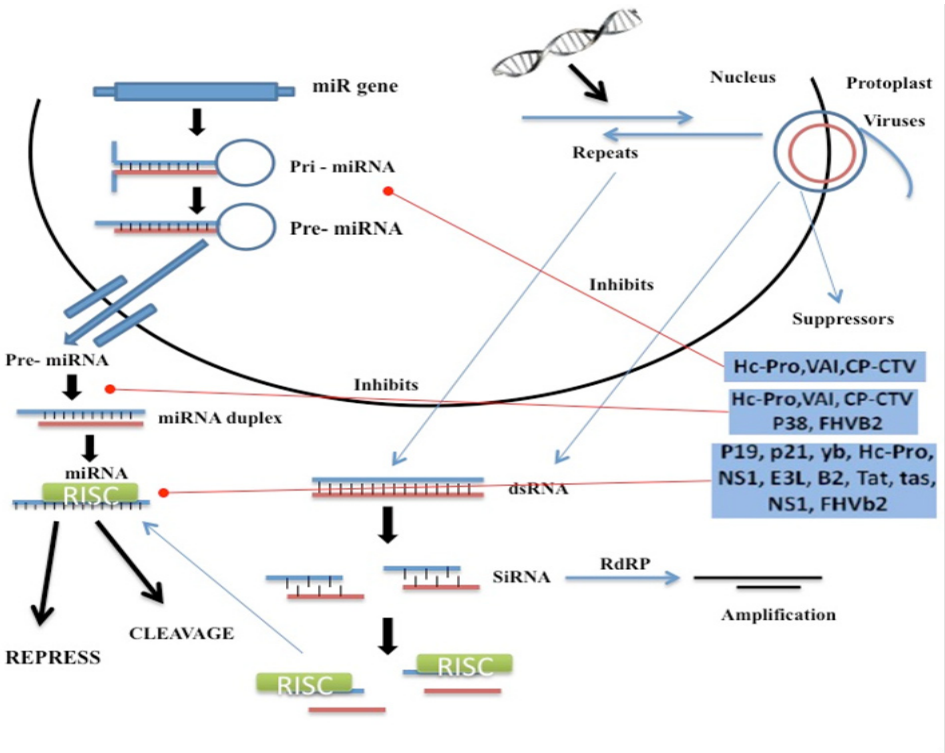

Figure 1: The different modes of interference of the known RNA silencing suppressors on miRNA and siRNA biogenesis pathway. The suppressors may interrupt the RNA silencing pathway at different steps from the beginning of biogenesis,small RNA maturation and loading into RISC.

6. Tables

Table 1: List of viruses used for the construction of VIGS vectors.

Virus Backbone	Reference
RNA Virus and their satellites	
Tobacco Mosaic Virus (TMV)	[43,52]
SatelliteTobaccoMosaic Virus (STMV)	[53]
Potato Virus X (PVX)	[37]
Tobacco Rattle Virus (TRV)	[44]
Barley Stripe Mosaic Virus (BSMV)W	[54,55]
Pea Early Browning Virus (PEBV)	[56]
Brome Mosaic Virus (BMV)	[57]
Bean Pod Mottle Virus	[58]
Cucumber Mosaic Virus (CMV)	[59]
Tomato Mosaic Virus (TMV)	[60]
DNA Virus	
Tomato Golden Mosaic Virus (TGMV)	[61]
Cabbage Leaf Curl Virus (CaLCV)	[62]
Tomato Leaf Curl Virus (ToLCV)	[51,63]
Tomato LeafCurl Virus satellite	[64]
Satellite DNAβ of Tomato Yellow Leaf Curl China Virus (TYLCV)	[65]
Tobacco Curly Shoot Virus	[66]

Table 2: List of suppressor molecules identified from different plant viruses.

Genome genome	Virus	Suppressor protein	Type of silencing mechanism	Reference
DNA	African cassava mosaic virus	AC2 AC4	Local Systemic	(68, 73)
	Tomato golden mosaic virus	AL2	-	(74)
	Tomato yellow leaf curl virus-C	C2	Local and Systemic	(75, 76)
	Beet curly top virus	L2	-	(74)
RNA	Turnip crinkle virus	CP (P38)	Local	(77, 78)
	Beet yellows virus	p21	Local	(79)
	Citrus tristeza virus	p20	Local	(80)
		p23	Local and Systemic	(80)
		CP	Systemic	(80)
	Beet yellow stunt virus	P22	Local	(79)
	Cowpea mosaic virus	S coat	Local	(68)
	Cucumber mosaic virus	2b	Local* and Systemic	(81, 82)
	Peanut clump virus	p15	Local and Systemic	(83)
	Barley stripe mosaic virus	γb	-	(84)
	Beet western yellows virus	P0	Local and not systemic	(85)
	Potato virus X	P25	Systemic	(86)
	Potato virus Y	HC-Pro	Local and Systemic*	(81, 87)
	Rice yellow mottle virus	P1	-	(68)
	Tomato mosaic virus	126-kDa protein	-	(68, 88)
		130-kDa protein	Local	(89)
	Tomato bushy stunt virus	P19	Local and Systemic (binds siRNAs)	(68, 90)
	Cymbidium ringspot virus	P19	Local and Systemic (binds siRNAs)	(91)
	Turnip yellow mosaic virus	p69	Local	(50)

7. Conclusions

In plants, the RNA-dependent silencing of gene function serves as a key regulatory mechanism forming a crucial link between defence, development and adaptation to stress. In response to virus attack it encompassesthe first line ofprotection for restricting accumulation or spread of invading viruses. The phenomenon of RNA silencing has enormous potential to be exploited as a tool to counter virus attack. As a counter defensive mechanism, the viruses encode the RSS proteins to replicate and establish in the host plants. The RSS have the ability to suppress the RNA silencing at different stages of the pathway andmutation in these proteins result in attenuated symptom development and mild disease manifestation. Thus, RNA silencing based strategies designed to inhibit the RSS activity can play a crucial rolein developing virus resistance. It is expected that further growth in knowledge will help in adapting more innovative designs to enhance the robustness of RNAi technology towards developing disease-free crop plants.

8. References

1. Anderson JP, Daifuku R, Loeb LA. Viral error catastrophe by mutagenic nucleosides. Annual Review in Microbiology 2004;58:183-205.

2. Agrawal N, Dasaradhi P, Mohmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK. RNA interference: biology, mechanism, and applications. Microbiology and Molecular Biology Reviews. 2003;67(4):657-685.

3. Sanan-Mishra N, Mukherjee SK. A peep into the plant miRNA world. Open Plant Science Journal. 2007;1:1-9.

4. Jorgensen RA, Que Q, Stam M. Do unintended antisense transcripts contribute to sense cosuppression in plants? Trends in Genetics. 1999;15(1):11-12.

5. De Haan P, Gielen JJ, Prins M, Wijkamp IG, van Schepen A, Peters D, et al. Characterization of RNA-mediated resistance to tomato spotted wilt virus in transgenic tobacco plants. Nature Biotechnology. 1992;10:1133-1137.

6. Lindbo JA, Dougherty WG. Untranslatable transcripts of the tobacco etch virus coat protein gene sequence can interfere with tobacco etch virus replication in transgenic plants and protoplasts. Virology. 1992;189(2):725-733.

7. Roth A, Breaker RR. Selection in vitro of allosteric ribozymes. In: Sioud M. (Ed) Ribozymes and siRNA Protocols: Methods in Molecular Biology, Springer; 2004. 252:145-164.

8. Hamilton AJ, Baulcombe DC. A species of small antisense RNA in posttranscriptional gene silencing in plants. Science. 1999;286(5441):950-952.

9. Peláez P, Sanchez F. Small RNAs in plant defense responses during viral and bacterial interactions: similarities and differences. Frontiers in Plant Science. 2013;4:343.

10. Sanan-Mishra N, Varanasi SP, Mukherjee SK. Micro-regulators of auxin action. Plant Cell Reports. 2013;32(6):733-740.

11. Szittya G, Burgyán J. RNA interference-mediated intrinsic antiviral immunity in plants.In: Cullen BR. (Ed) Current Topics in Microbiology and Immunology. Springer;2013. 371:153-181 .

12. Roth BM, Pruss GJ, Vance VB. Plant viral suppressors of RNA silencing. Virus Research. 2004;102(1):97-108.

13. Enquist L. Virology in the 21st century. Journal of Virology. 2009;83(11):5296-5308.

14. Uprichard SL. The therapeutic potential of RNA interference. FEBS Letters. 2005;579(26):5996-6007.

15. Davies KM. Genetic modification of plant metabolism for human health benefits. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2007;622(1):122-137.

16. Gu L, Knipple DC. Recent advances in RNA interference research in insects: Implications for future insect pest management strategies. Crop Protection. 2013;45:36-40.

17. Sindhu AS, Maier TR, Mitchum MG, Hussey RS, Davis EL, Baum TJ. Effective and specific in planta RNAi in cyst nematodes: expression interference of four parasitism genes reduces parasitic success. Journal of Experimental Botany. 2009;60(1):315-324.

18. Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature. 2000;404(6775):293-296

19. Elbashir SM, Lendeckel W, Tuschl T. RNA interference is mediated by 21- and 22-nucleotide RNAs. Genes and Development. 2001;15(2):188-200.

20. Hammond SM, Boettcher S, Caudy AA, Kobayashi R, Hannon GJ. Argonaute2, a link between genetic and biochemical analyses of RNAi. Science. 2001;293(5532):1146-1150.

21. Nykänen A, Haley B, Zamore PD. ATP requirements and small interfering RNA structure in the RNA interference pathway. Cell. 2001;107(3):309-321.

22. Tang G, Reinhart BJ, Bartel DP, Zamore PD. A biochemical framework for RNA silencing in plants. Genes and Development. 2003;17(1):49-63.

23. Mateos JL, Bologna NG, Palatnik JF. Biogenesis of plant microRNAs.In: Erdmann VA and Barciszewski J (Eds) Non Coding RNAs in Plants: Springer; 2011. p. 251-268.

24. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature. 1998;391(6669):806-811.

25. Wesley SV, Helliwell CA, Smith NA, Wang M, Rouse DT, Liu Q, et al. Construct design for efficient, effective and high-throughput gene silencing in plants. The Plant Journal. 2001;27(6):581-590.

26. Du TG, Jellbauer S, Müller M, Schmid M, Niessing D, Jansen RP. Nuclear transit of the RNA-binding protein She2 is required for translational control of localized ASH1 mRNA. EMBO Reports. 2008;9(8):781-787.

27. Schwab R, Voinnet O. miRNA processing turned upside down. The EMBO Journal. 2009;28(23):3633-3634.

28. Sanan-Mishra N, Karjee S, Mukherjee SK . Design and delivery of small RNAs for RNAi technology. Dynamic Biochemistry, Process Biotechnolgy and Molecular Biology 2010; 4(1):20.

29. Mateos JL, Bologna NG, Chorostecki U, Palatnik JF. Identification of microRNA processing determinants by random mutagenesis of Arabidopsis MIR172a precursor. Current Biology. 2010;20(1):49-54.

30. Vazquez F, Vaucheret H, Rajagopalan R, Lepers C, Gasciolli V, Mallory AC, et al. Endogenous trans-acting siRNAs regulate the accumulation of Arabidopsis mRNAs. Molecular Cell. 2004;16(1):69-79.

31. Pruss G, Ge X, Shi XM, Carrington JC, Vance VB. Plant viral synergism: the potyviral genome encodes a broad-range pathogenicity enhancer that transactivates replication of heterologous viruses. The Plant Cell. 1997;9(6):859-868.

32. Ratcliff FG, MacFarlane SA, Baulcombe DC. Gene silencing without DNA: RNA-mediated cross-protection between viruses. The Plant Cell. 1999;11(7):1207-1215.

33. Ratcliff F, Harrison BD, Baulcombe DC. A similarity between viral defense and gene silencing in plants. Science. 1997;276(5318):1558-1560.

34. Voinnet O. RNA silencing as a plant immune system against viruses. Trends in Genetics. 2001;17(8):449-459.

35. Szittya G, Molnár A, Silhavy D, Hornyik C, Burgyán J. Short defective interfering RNAs of tombusviruses are not targeted but trigger post-transcriptional gene silencing against their helper virus. The Plant Cell. 2002;14(2):359-372.

36. Van Kammen A. Virus-induced gene silencing in infected and transgenic plants. Trends in Plant Science. 1997;2(11):409-411.

37. Ruiz MT, Voinnet O, Baulcombe DC. Initiation and maintenance of virus-induced gene silencing. The Plant Cell. 1998;10(6):937-946.

38. Baulcombe DC. Fast forward genetics based on virus-induced gene silencing. Current Opinion in Plant Biology. 1999;2(2):109-113.

39. Kurihara Y, Watanabe Y. Arabidopsis micro-RNA biogenesis through Dicer-like 1 protein functions. Proceedings of the National Academy of Sciences USA. 2004;101(34):12753-12758.

40. Voinnet O. Origin, biogenesis, and activity of plant microRNAs. Cell. 2009;136(4):669-687.

41. Burch-Smith TM, Schiff M, Liu Y, Dinesh-Kumar SP. Efficient virus-induced gene silencing in Arabidopsis. Plant Physiology. 2006;142(1):21-27.

42. Lindbo JA, Silva-Rosales L, Proebsting WM, Dougherty WG. Induction of a highly specific antiviral state in transgenic plants: Implications for regulation of gene expression and virus resistance. The Plant Cell. 1993;5(12):1749-1759.

43. Kumagai M, Donson J, Della-Cioppa G, Harvey D, Hanley K, Grill L. Cytoplasmic inhibition of carotenoid biosynthesis with virus-derived RNA. Proceedings of the National Academy of Sciences USA. 1995;92(5):1679-1683.

44. Ratcliff F, Martin-Hernandez AM, Baulcombe DC. Technical advance: tobacco rattle virus as a vector for analysis of gene function by silencing. The Plant Journal. 2001;25(2):237-245.

45. Liu Y, Schiff M, Dinesh-Kumar S. Virus-induced gene silencing in tomato. The Plant Journal. 2002;31(6):777-786.

46. Burch-Smith TM, Anderson JC, Martin GB, Dinesh-Kumar SP. Applications and advantages of virus-induced gene silencing for gene function studies in plants. The Plant Journal. 2004;39(5):734-746.

47. Hileman LC, Drea S, Martino G, Litt A, Irish VF. Virus-induced gene silencing is an effective tool for assaying gene function in the basal eudicot species *Papaver somniferum* (opium poppy). The Plant Journal. 2005;44(2):334-341.

48. Wang X, El Naqa IM. Prediction of both conserved and nonconserved microRNA targets in animals. Bioinformatics. 2008;24(3):325-332.

49. Brigneti G, Martín-Hernández AM, Jin H, Chen J, Baulcombe DC, Baker B, et al. Virus-induced gene silencing in Solanum species. The Plant Journal. 2004;39(2):264-272.

50. Chen J, Li WX, Xie D, Peng JR, Ding SW. Viral virulence protein suppresses RNA silencing–mediated defense but upregulates the role of microRNA in host gene expression. The Plant Cell. 2004;16(5):1302-1313.

51. Pandey P, Choudhury NR, Mukherjee SK. A geminiviral amplicon (VA) derived from Tomato leaf curl virus (ToLCV) can replicate in a wide variety of plant species and also acts as a VIGS vector. Virology Journal 2009;6:152.

52. Lacomme C, Hrubikova K. Enhancement of virus-induced gene silencing through viral-based production of inverted-repeats. The Plant Journal. 2003;34(4):543-553.

53. Gosselé V, Faché I, Meulewaeter F, Cornelissen M, Metzlaff M. SVISS–a novel transient gene silencing system for gene function discovery and validation in tobacco plants. The Plant Journal. 2002;32(5):859-866.

54. Fitzmaurice WP, Holzberg S, Lindbo JA, Padgett HS, Palmer KE, Wolfe GM, et al. Epigenetic modification of plants with systemic RNA viruses. Omics: A Journal of Integrative Biology. 2002;6(2):137-151.

55. Holzberg S, Brosio P, Gross C, Pogue GP. Barley stripe mosaic virus-induced gene silencing in a monocot plant. The Plant Journal. 2002;30(3):315-327.

56. Constantin GD, Krath BN, MacFarlane SA, Nicolaisen M, Elisabeth Johansen I, Lund OS. Virus-induced gene silencing as a tool for functional genomics in a legume species. The Plant Journal. 2004;40(4):622-631.

57. Ding XS, Schneider WL, Chaluvadi SR, Mian MR, Nelson RS. Characterization of a Brome mosaic virus strain and its use as a vector for gene silencing in monocotyledonous hosts. Molecular Plant-Microbe Interactions. 2006;19(11):1229-1239.

58. Zhang C, Ghabrial SA. Development of Bean pod mottle virus-based vectors for stable protein expression and sequence-specific virus-induced gene silencing in soybean. Virology. 2006;344(2):401-411.

59. Sudarshana MR, Plesha MA, Uratsu SL, Falk BW, Dandekar AM, Huang TK, et al. A chemically inducible cucumber mosaic virus amplicon system for expression of heterologous proteins in plant tissues. Plant Biotechnology Journal. 2006;4(5):551-559.

60. Saejung W, Fujiyama K, Takasaki T, Ito M, Hori K, Malasit P, et al. Production of dengue 2 envelope domain III in plant using TMV-based vector system. Vaccine. 2007;25(36):6646-6654.

61. Peele C, Jordan CV, Muangsan N, Turnage M, Egelkrout E, Eagle P, et al. Silencing of a meristematic gene using geminivirus-derived vectors. The Plant Journal. 2001;27(4):357-366.

62. Turnage MA, Muangsan N, Peele CG, Robertson D. Geminivirus-based vectors for gene silencing in Arabidopsis. The Plant Journal. 2002;30(1):107-114.

63. Huang C, Xie Y, Zhou X. Efficient virus-induced gene silencing in plants using a modified geminivirus DNA1 component. Plant Biotechnology Journal. 2009;7(3):254-265.

64. Li D, Behjatnia SAA, Dry IB, Walker AR, Randles JW, Rezaian MA. Tomato leaf curl virus satellite DNA as a gene silencing vector activated by helper virus infection. Virus Research. 2008;136(1):30-34.

65. Tao X, Qing L, Zhou X. Function of A-Rich region in DNAβ associated with Tomato yellow leaf curl China virus. Chinese Science Bulletin. 2004;49(14):1490-1493.

66. Qian Y, Mugiira RB, Zhou X. A modified viral satellite DNA-based gene silencing vector is effective in association with heterologous begomoviruses. Virus Research. 2006;118(1):136-142.

67. Fofana IB, Sangaré A, Collier R, Taylor C, Fauquet CM. A geminivirus-induced gene silencing system for gene function validation in cassava. Plant Molecular Biology. 2004;56(4):613-624.

68. Voinnet O, Pinto YM, Baulcombe DC. Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. Proceedings of the National Academy of Sciences USA. 1999;96(24):14147-14152.

69. Shi B-J, Palukaitis P, Symons RH. Differential virulence by strains of Cucumber mosaic virus is mediated by the 2b gene. Molecular Plant-Microbe Interactions. 2002;15(9):947-955.

70. Hartitz MD, Sunter G, Bisaro DM. The tomato golden mosaic virus transactivator (TrAP) is a single-stranded DNA and zinc-binding phosphoprotein with an acidic activation domain. Virology. 1999;263(1):1-14.

71. Damirdagh IS, Ross AF. A marked synergistic interaction of potato viruses X and Y in inoculated leaves of tobacco. Virology. 1967;31(2):296-307.

72. Mlotshwa S, Verver J, Sithole-Niang I, Prins M, Van Kammen A, Wellink J. Transgenic plants expressing HC-Pro show enhanced virus sensitivity while silencing of the transgene results in resistance. Virus Genes. 2002;25(1):45-57.

73. Vanitharani R, Chellappan P, Pita JS, Fauquet CM. Differential roles of AC2 and AC4 of cassava geminiviruses in mediating synergism and suppression of posttranscriptional gene silencing. Journal of Virology. 2004;78(17):9487-9498.

74. Wang H, Hao L, Shung C-Y, Sunter G, Bisaro DM. Adenosine kinase is inactivated by geminivirus AL2 and L2 proteins. The Plant Cell. 2003;15(12):3020-3032.

75. Dong X, van Wezel R, Stanley J, Hong Y. Functional characterization of the nuclear localization signal for a suppressor of posttranscriptional gene silencing. Journal of Virology. 2003;77(12):7026-7033.

76. van Wezel R, Liu H, Wu Z, Stanley J, Hong Y. Contribution of the zinc finger to zinc and DNA binding by a suppressor of posttranscriptional gene silencing. Journal of Virology. 2003;77(1):696-700.

77. Qu F, Ren T, Morris TJ. The coat protein of turnip crinkle virus suppresses posttranscriptional gene silencing at an early initiation step. Journal of Virology. 2003;77(1):511-522.

78. Thomas CL, Leh V, Lederer C, Maule AJ. Turnip crinkle virus coat protein mediates suppression of RNA silencing in Nicotiana benthamiana. Virology. 2003;306(1):33-41.

79. Reed JC, Kasschau KD, Prokhnevsky AI, Gopinath K, Pogue GP, Carrington JC, et al. Suppressor of RNA silencing encoded by Beet yellows virus. Virology. 2003;306(2):203-209.

80. Lu R, Folimonov A, Shintaku M, Li W-X, Falk BW, Dawson WO, et al. Three distinct suppressors of RNA silencing encoded by a 20-kb viral RNA genome. Proceedings of the National Academy of Sciences USA. 2004;101(44):15742-15747.

81. Brigneti G, Voinnet O, Li WX, Ji LH, Ding SW, Baulcombe DC. Viral pathogenicity determinants are suppressors of transgene silencing in *Nicotiana benthamiana*. The EMBO Journal. 1998;17(22):6739-6746.

82. Lucy AP, Guo HS, Li WX, Ding SW. Suppression of post-transcriptional gene silencing by a plant viral protein

localized in the nucleus. The EMBO Journal. 2000;19(7):1672-1680.

83. Dunoyer P, Pfeffer S, Fritsch C, Hemmer O, Voinnet O, Richards KE. Identification, subcellular localization and some properties of a cysteine-rich suppressor of gene silencing encoded by peanut clump virus. The Plant Journal. 2002;29(5):555-567.

84. Yelina NE, Savenkov EI, Solovyev AG, Morozov SY, Valkonen JP. Long-distance movement, virulence, and RNA silencing suppression controlled by a single protein in hordei-and potyviruses: complementary functions between virus families. Journal of Virology. 2002;76(24):12981-12991.

85. Pfeffer S, Dunoyer P, Heim F, Richards K, Jonard G, Ziegler-Graff V. P0 of beet Western yellows virus is a suppressor of posttranscriptional gene silencing. Journal of Virology. 2002;76(13):6815-6824.

86. Voinnet O, Lederer C, Baulcombe DC. A viral movement protein prevents spread of the gene silencing signal in *Nicotiana benthamiana*. Cell. 2000;103(1):157-167.

87. Anandalakshmi R, Pruss GJ, Ge X, Marathe R, Mallory AC, Smith TH, et al. A viral suppressor of gene silencing in plants. Proceedings of the National Academy of Sciences USA. 1998;95(22):13079-13084.

88. Ding XS, Liu J, Cheng N-H, Folimonov A, Hou Y-M, Bao Y, et al. The Tobacco mosaic virus 126-kDa protein associated with virus replication and movement suppresses RNA silencing. Molecular Plant-Microbe Interactions. 2004;17(6):583-592.

89. Kubota K, Tsuda S, Tamai A, Meshi T. Tomato mosaic virus replication protein suppresses virus-targeted posttranscriptional gene silencing. Journal of Virology. 2003;77(20):11016-11026.

90. Lakatos L, Szittya G, Silhavy D, Burgyán J. Molecular mechanism of RNA silencing suppression mediated by p19 protein of tombusviruses. The EMBO Journal. 2004;23(4):876-884.

91. Szittya G, Silhavy D, Dalmay T, Burgyán J. Size-dependent cell-to-cell movement of defective interfering RNAs of Cymbidium ringspot virus. Journal of General Virology. 2002;83(6):1505-1510.

92. Kasschau KD, Carrington JC. A counterdefensive strategy of plant viruses: suppression of posttranscriptional gene silencing. Cell. 1998;95(4):461-470.

93. Delgadillo MO, Sáenz P, Salvador B, García JA, Simón-Mateo C. Human influenza virus NS1 protein enhances viral pathogenicity and acts as an RNA silencing suppressor in plants. Journal of General Virology. 2004;85(4):993-999.

94. Li W-X, Li H, Lu R, Li F, Dus M, Atkinson P, et al. Interferon antagonist proteins of influenza and vaccinia viruses are suppressors of RNA silencing. Proceedings of the National Academy of Sciences USA. 2004;101(5):1350-1355.

95. Li H, Li WX, Ding SW. Induction and suppression of RNA silencing by an animal virus. Science. 2002;296(5571):1319-1321.

96. Karjee S, Minhas A, Sood V, Ponia SS, Banerjea AC, Chow VT, et al. The 7a accessory protein of severe acute respiratory syndrome coronavirus acts as an RNA silencing suppressor. Journal of Virology. 2010;84(19):10395-10401.

97. Elmayan T, Vaucheret H. Expression of single copies of a strongly expressed 35S transgene can be silenced post-transcriptionally. The Plant Journal. 1996;9(6):787-797.

98. Voinnet O, Baulcombe DC. Systemic signalling in gene silencing. Nature. 1997;389:553.

99. Voinnet O, Vain P, Angell S, Baulcombe DC. Systemic spread of sequence-specific transgene RNA degradation in plants is initiated by localized introduction of ectopic promoterless DNA. Cell. 1998;95(2):177-187.

100. Llave C, Kasschau KD, Carrington JC. Virus-encoded suppressor of posttranscriptional gene silencing targets a maintenance step in the silencing pathway. Proceedings of the National Academy of Sciences USA. 2000;97(24):13401-13406.

101. Johansen LK, Carrington JC. Silencing on the spot. Induction and suppression of RNA silencing in the Agrobacterium-mediated transient expression system. Plant Physiology. 2001;126(3):930-938.

102. Kasschau KD, Xie Z, Allen E, Llave C, Chapman EJ, Krizan KA, et al. P1/HC-Pro, a viral suppressor of RNA silencing, interferes with Arabidopsis development and miRNA function. Developmental Cell. 2003;4(2):205-217.

103. Chapman EJ, Prokhnevsky AI, Gopinath K, Dolja VV, Carrington JC. Viral RNA silencing suppressors inhibit the microRNA pathway at an intermediate step. Genes and Development. 2004;18(10):1179-1186.

104. Dunoyer P, Lecellier C-H, Parizotto EA, Himber C, Voinnet O. Probing the microRNA and small interfering RNA pathways with virus-encoded suppressors of RNA silencing. The Plant Cell. 2004;16(5):1235-1250.

105. Anandalakshmi R, Marathe R, Ge X, Herr J, Mau C, Mallory A, et al. A calmodulin-related protein that suppresses posttranscriptional gene silencing in plants. Science. 2000;290(5489):142-144.

106. Mallory AC, Ely L, Smith TH, Marathe R, Anandalakshmi R, Fagard M, et al. HC-Pro suppression of transgene silencing eliminates the small RNAs but not transgene methylation or the mobile signal. The Plant Cell. 2001;13(3):571-583.

107. Guo HS, Ding SW. A viral protein inhibits the long range signaling activity of the gene silencing signal. The EMBO Journal. 2002;21(3):398-407.

108. Carrington JC, Kasschau KD, Johansen LK. Activation and suppression of RNA silencing by plant viruses. Virology. 2001;281(1):1-5.

109. Li WX, Ding SW. Viral suppressors of RNA silencing. Current Opinion in Biotechnology. 2001;12(2):150-154.

110. Baulcombe DC. RNA silencing. Current Biology. 2002;12(3):R82-R4.

111. Moissiard G, Voinnet O. Viral suppression of RNA silencing in plants. Molecular Plant Pathology. 2004;5(1):71-82.

112. Roth A, Breaker RR. Selection in vitro of allosteric ribozymes.Ribozymes and siRNA Protocols: Springer; 2004. p. 145-164.

113. Silhavy D, Burgyán J. Effects and side-effects of viral RNA silencing suppressors on short RNAs. Trends in Plant Science. 2004;9(2):76-83.

114. Mérai Z, Kerényi Z, Kertész S, Magna M, Lakatos L, Silhavy D. Double-stranded RNA binding may be a general plant RNA viral strategy to suppress RNA silencing. Journal of Virology. 2006;80(12):5747-5756.

115. Vargason JM, Szittya G, Burgyán J, Hall TMT. Size selective recognition of siRNA by an RNA silencing suppressor. Cell. 2003;115(7):799-811.

116. Ye K, Malinina L, Patel DJ. Recognition of small interfering RNA by a viral suppressor of RNA silencing. Nature. 2003;426(6968):874-878.

117. Ye K, Patel DJ. RNA silencing suppressor p21 of Beet yellows virus forms an RNA binding octameric ring structure. Structure. 2005;13(9):1375-1384.

118. Lakatos L, Csorba T, Pantaleo V, Chapman EJ, Carrington JC, Liu YP, et al. Small RNA binding is a common strategy to suppress RNA silencing by several viral suppressors. The EMBO Journal. 2006;25(12):2768-2780.

119. Goto K, Kobori T, Kosaka Y, Natsuaki T, Masuta C. Characterization of silencing suppressor 2b of cucumber mosaic virus based on examination of its small RNA-binding abilities. Plant and Cell Physiology. 2007;48(7):1050-1060.

120. Singh G, Popli S, Hari Y, Malhotra P, Mukherjee S, Bhatnagar RK. Suppression of RNA silencing by Flock house virus B2 protein is mediated through its interaction with the PAZ domain of Dicer. The FASEB Journal. 2009;23(6):1845-1857.

121. Llave C, Kasschau KD, Rector MA, Carrington JC. Endogenous and silencing-associated small RNAs in plants.

The Plant Cell. 2002;14(7):1605-1619.

122. Mallory AC, Reinhart BJ, Bartel D, Vance VB, Bowman LH. A viral suppressor of RNA silencing differentially regulates the accumulation of short interfering RNAs and micro-RNAs in tobacco. Proceedings of the National Academy of Sciences USA. 2002;99(23):15228-15233.

123. Park W, Li J, Song R, Messing J, Chen X. CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in Arabidopsis thaliana. Current Biology. 2002;12(17):1484-1495.

124. Alvarez JP, Pekker I, Goldshmidt A, Blum E, Amsellem Z, Eshed Y. Endogenous and synthetic microRNAs stimulate simultaneous, efficient, and localized regulation of multiple targets in diverse species. The Plant Cell. 2006;18(5):1134-1151.

125. Siddiqui SA, Sarmiento C, Truve E, Lehto H, Lehto K. Phenotypes and functional effects caused by various viral RNA silencing suppressors in transgenic Nicotiana benthamiana and N. tabacum. Molecular Plant-Microbe Interactions. 2008;21(2):178-187.

126. Chen HY, Yang J, Lin C, Yuan YA. Structural basis for RNA-silencing suppression by Tomato aspermy virus protein 2b. EMBO Reports. 2008;9(8):754-760.

127. Deleris A, Gallego-Bartolome J, Bao J, Kasschau KD, Carrington JC, Voinnet O. Hierarchical action and inhibition of plant Dicer-like proteins in antiviral defense. Science. 2006;313(5783):68-71.

128. Haas G, Azevedo J, Moissiard G, Geldreich A, Himber C, Bureau M, et al. Nuclear import of CaMV P6 is required for infection and suppression of the RNA silencing factor DRB4. The EMBO Journal. 2008;27(15):2102-2112.

129. Kumar V, Mishra SK, Rahman J, Taneja J, Sundaresan G, Sanan-Mishra N, et al. Mungbean yellow mosaic Indian virus encoded AC2 protein suppresses RNA silencing by inhibiting Arabidopsis RDR6 and AGO1 activities. Virology. 2015;486:158-172.

130. Bortolamiol D, Pazhouhandeh M, Ziegler-Graff V. Viral suppression of RNA silencing by destabilization of ARGONAUTE 1. Plant Signaling and Behavior. 2008;3(9):657-659.

131. Ruiz-Ferrer V, Voinnet O. Viral suppression of RNA silencing: 2b wins the Golden Fleece by defeating Argonaute. Bioessays. 2007;29(4):319-323.

132. Duan CG, Fang YY, Zhou BJ, Zhao JH, Hou WN, Zhu H, et al. Suppression of Arabidopsis ARGONAUTE1-mediated slicing, transgene-induced RNA silencing, and DNA methylation by distinct domains of the Cucumber mosaic virus 2b protein. The Plant Cell. 2012;24(1):259-274.

134. Buchmann RC, Asad S, Wolf JN, Mohannath G, Bisaro DM. Geminivirus AL2 and L2 Proteins Suppress Transcriptional Gene Silencing and Cause Genome-Wide Reductions in Cytosine Methylation. Journal of Virology. 2009;83(10):5005-5013.

133. Trinks D, Rajeswaran R, Shivaprasad P, Akbergenov R, Oakeley EJ, Veluthambi K, et al. Suppression of RNA silencing by a geminivirus nuclear protein, AC2, correlates with transactivation of host genes. Journal of Virology. 2005;79(4):2517-2527.

135. Yang X, Xie Y, Raja P, Li S, Wolf JN, Shen Q, et al. Suppression of methylation-mediated transcriptional gene silencing by betaC1-SAHH protein interaction during geminivirus-betasatellite infection. PLoS Pathogens. 2011;7(10):e1002329.

136. Zhang Z, Chen H, Huang X, Xia R, Zhao Q, Lai J, et al. BSCTV C2 attenuates the degradation of SAMDC1 to suppress DNA methylation-mediated gene silencing in Arabidopsis. The Plant Cell. 2011;23(1):273-288.

137. Sanford J, Johnston S. The concept of parasite-derived resistance—Deriving resistance genes from the parasite's own genome. Journal of Theoretical Biology. 1985;113(2):395-405.

139. Beachy RN. Mechanisms and applications of pathogen-derived resistance in transgenic plants. Current Opinion in Biotechnology. 1997;8(2):215-220.

138. Wilson TM. Strategies to protect crop plants against viruses: pathogen-derived resistance blossoms. Proceedings of the National Academy of Sciences USA. 1993;90(8):3134-3141.

140. Prins M, Goldbach R. RNA-mediated virus resistance in transgenic plants. Archives of Virology. 1996;141(12):2259-76.

141. Beachy RN. Coat-protein-mediated resistance to tobacco mosaic virus: discovery mechanisms and exploitation. Philosophical Transactions of the Royal Society B: Biological Sciences. 1999;354(1383):659-664.

142. Abel PP, Nelson RS, De B, Hoffmann N, Rogers SG, Fraley RT, et al. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science. 1986;232(4751):738-743.

143. Hayakawa T, Zhu Y, Itoh K, Kimura Y, Izawa T, Shimamoto K, et al. Genetically engineered rice resistant to rice stripe virus, an insect-transmitted virus. Proceedings of the National Academy of Sciences USA. 1992;89(20):9865-9869.

144. Farinelli L, Malnoë P. Coat Protein Gene-Mediated Resistance to Potato Virus Y in Tobacco: Examination of the resistance mechanisms-is the transgenic coat protein required for protection? Molecular Plant Microbe Interactions. 1993;6:284-292.

145. Kunik T, Salomon R, Zamir D, Navot N, Zeidan M, Michelson I, et al. Transgenic tomato plants expressing the tomato yellow leaf curl virus capsid protein are resistant to the virus. Nature Biotechnology. 1994;12(5):500-504.

146. Chowrira G, Cavileer T, Gupta S, Lurquin P, Berger P. Coat protein-mediated resistance to pea enation mosaic virus in transgenic *Pisum Sativum* L. Transgenic research. 1998;7(4):265-271.

147. Germundsson A, Sandgren M, Barker H, Savenkov EI, Valkonen JP. Initial infection of roots and leaves reveals different resistance phenotypes associated with coat protein gene-mediated resistance to Potato mop-top virus. Journal of General Virology. 2002;83(5):1201-1209.

148. Srivastava A, Raj SK. Coat protein-mediated resistance against an Indian isolate of the Cucumber mosaic virus subgroup IB in Nicotiana benthamiana. Journal of Bioscience. 2008;33(2):249-257.

149. Liu B, Preisser EL, Chu D, Pan H, Xie W, Wang S, et al. Multiple forms of vector manipulation by a plant-infecting virus: Bemisia tabaci and tomato yellow leaf curl virus. Journal of Virology. 2013;87(9):4929-4937.

150. Höhnle M, Höfer P, Bedford ID, Briddon RW, Markham PG, Frischmuth T. Exchange of three amino acids in the coat protein results in efficient whitefly transmission of a nontransmissible Abutilon mosaic virus isolate. Virology. 2001;290(1):164-171.

151. Hanley-Bowdoin L, Elmer JS, Rogers SG. Expression of functional replication protein from tomato golden mosaic virus in transgenic tobacco plants. Proceedings of the National Academy of Sciences. 1990;87(4):1446-1450.

152. Noris E, Accotto GP, Tavazza R, Brunetti A, Crespi S, Tavazza M. Resistance to Tomato Yellow Leaf Curl Geminivirus inNicotiana benthamianaPlants Transformed with a Truncated Viral C1 Gene. Virology. 1996;224(1):130-138.

153. Brunetti A, Tavazza M, Noris E, Tavazza R, Caciagli P, Ancora G, et al. High Expression of Truncated Viral Rep Protein Confers Resistance to Tomato Yellow Leaf Curl Virus in Transgenic Tomato Plants. Molecular Plant-Microbe Interactions. 1997;10(5):571-579.

154. Chatterji A, Beachy RN, Fauquet CM. Expression of the oligomerization domain of the replication-associated protein (Rep) of Tomato leaf curl New Delhi virus interferes with DNA accumulation of heterologous geminiviruses. Journal of Biological Chemistry. 2001;276(27):25631-25638.

155. Duan Y-P, Powell C, Webb S, Purcifull D, Hiebert E. Geminivirus resistance in transgenic tobacco expressing mutated BC1 protein. Molecular Plant-Microbe Interactions. 1997a;10(5):617-623.

156. Duan Y-P, Powell CA, Purcifull DE, Broglio P, Hiebert E. Phenotypic variation in transgenic tobacco expressing mutated geminivirus movement/pathogenicity (BC1) proteins. Molecular Plant-Microbe Interactions. 1997b;10(9):1065-

1074.

157. Vanderschuren H, Akbergenov R, Pooggin MM, Hohn T, Gruissem W, Zhang P. Transgenic cassava resistance to African cassava mosaic virus is enhanced by viral DNA-A bidirectional promoter-derived siRNAs. Plant Molecular Biology. 2007;64(5):549-557.

158. Hou Y-M, Sanders R, Ursin VM, Gilbertson RL. Transgenic plants expressing geminivirus movement proteins: abnormal phenotypes and delayed infection by Tomato mottle virus in transgenic tomatoes expressing the Bean dwarf mosaic virus BV1 or BC1 proteins. Molecular Plant-Microbe Interactions. 2000;13(3):297-308.

159. Simons RW. Naturally occurring antisense RNA control—a brief review. Gene. 1988;72(1):35-44.

160. Izant JG, Weintraub H. Inhibition of thymidine kinase gene expression by anti-sense RNA: a molecular approach to genetic analysis. Cell. 1984;36(4):1007-1015.

161. Mol J, Van der Krol A, Van Tunen A, Van Blokland R, De Lange P, Stuitje A. Regulation of plant gene expression by antisense RNA. FEBS Letters. 1990;268(2):427-430.

162. Ecker JR, Davis RW. Inhibition of gene expression in plant cells by expression of antisense RNA. Proceedings of the National Academy of Sciences USA. 1986;83(15):5372-5376.

163. Van der Krol AR, Lenting PE, Veenstra J, van der Meer IM, Koes RE, Gerats AG, et al. An anti-sense chalcone synthase gene in transgenic plants inhibits flower pigmentation. Nature. 1988;333(6176):866-869.

164. Samac DA, Shah DM. Effect of chitinase antisense RNA expression on disease susceptibility of Arabidopsis plants. Plant Molecular Biology. 1994;25(4):587-596.

165. Sheehy RE, Kramer M, Hiatt WR. Reduction of polygalacturonase activity in tomato fruit by antisense RNA. Proceedings of the National Academy of Sciences USA. 1988;85(23):8805-8809.

166. Smith C, Watson C, Bird C, Ray J, Schuch W, Grierson D. Expression of a truncated tomato polygalacturonase gene inhibits expression of the endogenous gene in transgenic plants. Molecular and General Genetics. 1990;224(3):477-481.

167. Kawchuk L, Martin R, McPherson J. Sense and antisense RNA-mediated resistance to potato leafroll virus in Russet Burbank potato plants. Mol Plant-Microbe Interaction. 1991;4:247-253.

168. Day AG, Bejarano ER, Buck KW, Burrell M, Lichtenstein CP. Expression of an antisense viral gene in transgenic tobacco confers resistance to the DNA virus tomato golden mosaic virus. Proceedings of the National Academy of Science USA. 1991;88(15):6721-6725.

169. Asad S, Haris W, Bashir A, Zafar Y, Malik K, Malik N, et al. Transgenic tobacco expressing geminiviral RNAs are resistant to the serious viral pathogen causing cotton leaf curl disease. Archives of Virology. 2003;148(12):2341-2352.

170. Beachy RN, Loesch-Fries S, Tumer NE. Coat protein-mediated resistance against virus infection. Annual Review of Phytopathology. 1990;28(1):451-472.

171. Baulcombe DC. Mechanisms of pathogen-derived resistance to viruses in transgenic plants. The Plant Cell. 1996;8(10):1833.

172. Bendahmane M, Gronenborn B. Engineering resistance against tomato yellow leaf curl virus (TYLCV) using antisense RNA. Plant Molecular Biology. 1997;33(2):351-357.

173. Yang Y, Sherwood T, Patte C, Hiebert E, Polston J. Use of Tomato yellow leaf curl virus (TYLCV) Rep gene sequences to engineer TYLCV resistance in tomato. Phytopathology. 2004;94(5):490-496.

174. Watson JM, Fusaro AF, Wang M, Waterhouse PM. RNA silencing platforms in plants. FEBS Letters. 2005;579(26):5982-5987.

175. de Felippes FF, Wang Jw, Weigel D. MIGS: miRNA-induced gene silencing. The Plant Journal. 2012;70(3):541-

547.

176. Zrachya A, Kumar PP, Ramakrishnan U, Levy Y, Loyter A, Arazi T, et al. Production of siRNA targeted against TYLCV coat protein transcripts leads to silencing of its expression and resistance to the virus. Transgenic Research. 2007;16(3):385-398.

177. Reyes CA, De Francesco A, Peña EJ, Costa N, Plata MI, Sendin L, et al. Resistance to Citrus psorosis virus in transgenic sweet orange plants is triggered by coat protein–RNA silencing. Journal of Biotechnology. 2011;151(1):151-158.

178. Shekhawat UK, Ganapathi TR, Hadapad AB. Transgenic banana plants expressing small interfering RNAs targeted against viral replication initiation gene display high-level resistance to banana bunchy top virus infection. Journal of General Virology. 2012;93(Pt 8):1804-1813.

179. Wang MB, Abbott DC, Waterhouse PM. A single copy of a virus-derived transgene encoding hairpin RNA gives immunity to barley yellow dwarf virus. Molecular Plant Pathology. 2000;1(6):347-356.

180. Stanley J, Frischmuth T, Ellwood S. Defective viral DNA ameliorates symptoms of geminivirus infection in transgenic plants. Proceedings of the National Academy of Sciences USA. 1990;87(16):6291-6295.

181. Aragão FJ, Faria JC. First transgenic geminivirus-resistant plant in the field. Nature Biotechnology. 2009;27(12):1086-1088.

182. Koch A, Biedenkopf D, Furch A, Weber L, Rossbach O, Abdellatef E, et al. An RNAi-based control of Fusarium graminearum infections through spraying of long dsRNAs involves a plant passage and is controlled by the fungal silencing machinery. PLoS Pathogens.2016.12(10): e100590.

183. Chen W, Kastner C, Nowara D, Oliveira-Garcia E, Rutten T, Zhao Y, et al. Host-induced silencing of Fusarium culmorum genes protects wheat from infection. Journal of Experimental Botany. 2016. 67(17): 4979-4991.

184. Tenllado F, Barajas D, Vargas M, Atencio F, González-Jara P, Díaz-Ruíz J. Transient expression of homologous hairpin RNA causes interference with plant virus infection and is overcome by a virus encoded suppressor of gene silencing. Molecular Plant-Microbe Interactions. 2003a;16(2):149-158.

185. Tenllado F, Martínez-García B, Vargas M, Díaz-Ruíz JR. Crude extracts of bacterially expressed dsRNA can be used to protect plants against virus infections. BMC Biotechnology. 2003b;3(1):3.

186. Vanitharani R, Chellappan P, Fauquet CM. Short interfering RNA-mediated interference of gene expression and viral DNA accumulation in cultured plant cells. Proceedings of the National Academy of Sciences USA. 2003;100(16):9632-9636.

187. Abhary M, Anfoka G, Nakhla M, Maxwell D. Post-transcriptional gene silencing in controlling viruses of the Tomato yellow leaf curl virus complex. Archives of Virology. 2006;151(12):2349-2363.

188. Lennefors B-L, Savenkov EI, Bensefelt J, Wremerth-Weich E, van Roggen P, Tuvesson S, et al. dsRNA-mediated resistance to Beet Necrotic Yellow Vein Virus infections in sugar beet (Beta vulgaris L. ssp. vulgaris). Molecular Breeding. 2006;18(4):313-325.

189. Ramesh S, Mishra A, Praveen S. Hairpin RNA-mediated strategies for silencing of tomato leaf curl virus AC1 and AC4 genes for effective resistance in plants. Oligonucleotides. 2007;17(2):251-257.

190. Beale G, Hollins AJ, Benboubetra M, Sohail M, Fox SP, Benter I, et al. Gene silencing nucleic acids designed by scanning arrays: anti-EGFR activity of siRNA, ribozyme and DNA enzymes targeting a single hybridization-accessible region using the same delivery system. Journal of Drug Targeting. 2003;11(7):449-456.

191. Bumcrot D, Manoharan M, Koteliansky V, et al. RNAi therapeutics: a potential new class of pharmaceutical drugs. Nature Chemical Biology. 2006;2(12):711-719.

192. Paddison PJ, Caudy AA, Bernstein E, Hannon GJ, et al. Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells. Genes andDevelopment. 2002;16(8):948-958.

193. Yu JY, DeRuiter SL, Turner DL. RNA interference by expression of short-interfering RNAs and hairpin RNAs in mammalian cells. Proceedings of the National Academy of Sciences USA.2002;99(9):6047-6052.

194. Petrov N, Teneva A, Stoyanova M, Andonova R, Denev I, Tomlekova N. Blocking the systemic spread of potato virus Y in the tissues of potatoes by posttranscriptional gene silencing. Bulgarian Journal of Agricultural Science. 2015;21(2):288-294.

195. Hannon GJ. RNA interference. Nature. 2002;418(6894):244-251.

196. Seemanpillai M, Dry I, Randles J, Rezaian A. Transcriptional silencing of geminiviral promoter-driven transgenes following homologous virus infection. Molecular Plant-Microbe Interactions. 2003;16(5):429-438.

197. Akbergenov R, Si-Ammour A, Blevins T, Amin I, Kutter C, Vanderschuren H, et al. Molecular characterization of geminivirus-derived small RNAs in different plant species. Nucleic Acids Research. 2006;34(2):462-471.

198. Pooggin M, Shivaprasad P, Veluthambi K, Hohn T. RNAi targeting of DNA virus in plants. Nature Biotechnology. 2003;21(2):131-132.

199. Ossowski S, Schwab R, Weigel D. Gene silencing in plants using artificial microRNAs and other small RNAs. The Plant Journal. 2008;53(4):674-690.

200. Sablok G, Pérez-Quintero ÁL, Hassan M, Tatarinova TV, López C. Artificial microRNAs (amiRNAs) engineering–On how microRNA-based silencing methods have affected current plant silencing research. Biochemical and Biophysical Research Communications. 2011;406(3):315-319.

201. Zeng Y, Cullen BR. RNA interference in human cells is restricted to the cytoplasm. RNA. 2002;8(7):855-860.

202. Parizotto EA, Dunoyer P, Rahm N, Himber C, Voinnet O. In vivo investigation of the transcription, processing, endonucleolytic activity, and functional relevance of the spatial distribution of a plant miRNA. Genes and Development. 2004;18(18):2237-2242.

203. Schwab R, Ossowski S, Riester M, Warthmann N, Weigel D. Highly specific gene silencing by artificial microRNAs in Arabidopsis. The Plant Cell. 2006;18(5):1121-1133.

204. Reynolds A, Leake D, Boese Q, Scaringe S, Marshall WS, Khvorova A. Rational siRNA design for RNA interference. Nature Biotechnology. 2004;22(3):326-330.

205. Mallory AC, Reinhart BJ, Jones-Rhoades MW, Tang G, Zamore PD, Barton MK, et al. MicroRNA control of PHABULOSA in leaf development: importance of pairing to the microRNA 5′ region. The EMBO Journal. 2004;23(16):3356-3364.

206. Niu Q-W, Lin S-S, Reyes JL, Chen K-C, Wu H-W, Yeh S-D, et al. Expression of artificial microRNAs in transgenic Arabidopsis thaliana confers virus resistance. Nature Biotechnology. 2006;24(11):1420-1428

207. Liang Z, Wu H, Reddy S, Zhu A, Wang S, Blevins D, et al. Blockade of invasion and metastasis of breast cancer cells via targeting CXCR4 with an artificial microRNA. Biochemical and Biophysical Research Communications. 2007;363(3):542-546.

208. Qu J, Ye J, Fang R. Artificial microRNA-mediated virus resistance in plants. Journal of Virology. 2007;81(12):6690-6699.

209. Kim Y-S, Ke F, Lei X-Y, Zhu R, Zhang Q-Y. Viral envelope protein 53R gene highly specific silencing and iridovirus resistance in fish cells by AmiRNA. PloS One. 2010;5(4):e10308.

210. Ai T, Zhang L, Gao Z, Zhu C, Guo X. Highly efficient virus resistance mediated by artificial microRNAs that target the suppressor of PVX and PVY in plants. Plant Biology. 2011;13(2):304-316.

211. Gao Y-F, Yu L, Wei W, Li J-B, Luo Q-L, Shen J-L. Inhibition of hepatitis B virus gene expression and replication by artificial microRNA. World Journal of Gastroenterology: World Journal of Gastroenterology. 2008; 14(29):4684–4689.. 2008;14(29):4684.

212. Tiwari M, Sharma D, Trivedi PK. Artificial microRNA mediated gene silencing in plants: progress and perspectives. Plant Molecular Biology. 2014;86(1-2):1-18.

213. Yadava P, Suyal G, Mukherjee SK. Begomovirus DNA replication and pathogenicity. Current Science. 2010;98(3):360-368.

214. Tien VV, Choudhury NR, Mukherjee SK. Transgenic tomato plants expressing artificial microRNAs for silencing the pre-coat and coat proteins of a begomovirus, Tomato leaf curl New Delhi virus, show tolerance to virus infection. Virus Research. 2013;172(1):35-45.

215. Kung YJ, Lin SS, Huang YL, Chen TC, Harish SS, Chua NH, et al. Multiple artificial microRNAs targeting conserved motifs of the replicase gene confer robust transgenic resistance to negative-sense single-stranded RNA plant virus. Molecular Plant Pathology. 2012;13(3):303-317.

216. Singh R, Rai N, Singh M, Singh S, Srivastava K. Selection of tomato genotypes resistant to tomato leaf curl virus disease using biochemical and physiological markers. The Journal of Agricultural Science. 2015;153(04):646-655.