



REVIEW ARTICLE

RNA Interference: A Novel Technology for Virus Disease Management in Crop Plants

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ABSTRACT

RNAs play a significant role in regulating gene expression and their principal areas have been exploited for the control of plant viruses by the discovery of RNA silencing mechanism. RNA silencing or RNA interference (RNAi) is an innovative mechanism that regulates and restricts the amount of transcripts either by suppressing transcription (TGS) or by the degradation of sequence-specific RNA. RNAi can be used effectively to study the role of genes in a variety of eukaryotic organisms by reverse genetics. The technology has been employed in several fields such as drug resistance, therapeutics, development of genetically modified animals for research and transgenic plants targeting plant viruses. In plants, small interfering RNAs (siRNA) are the characteristic of 21 to 22 bp long dsRNA, which has been recognized by the regulatory mechanism of RNAi and leads to the sequence-specific degradation of target mRNA. In addition to virus disease control, RNAi can also be used to control mycotoxins and plant diseases caused by other organisms. This review enhances our current knowledge of RNAi and its larger applications in agriculture, specifically in plant virus disease management.

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INTRODUCTION

Plant pathogens are a significant threat for the cultivation of crops and it creates difficulties in agriculture production, especially the plant viruses cause significant loss in the productivity of economically important crops around the world. The virus infection on crop plants causes 50-100% yield reduction depending upon the nature of crops and stages of virus infection. The complete yield loss was also noticed in several crops if it is infected during the early stages of infection and believed to cause one-third global loss of crop production. The strategies for viral disease management are based on agricultural practices, such as application of pesticides to manage the virus transmitting insect vectors, planting the fast-growing cereals as barrier crops to prevent insect movement, destroying the vulnerable weed hosts, and conserving optimal plant density to reduce disease incidence. However, they are rarely practiced under subsistence agriculture systems. In the past, traditional methods like use of cross protection and increased host plant resistance through beneficial microbes have been followed for virus disease management in several crops and that have worked markedly to some extent. Later, genes that are available naturally have been explored as one of the most effective sources to confer resistance

in plants. However, only limited naturally available genes were explored to develop resistant varieties through molecular and breeding programmes (Sasaya *et al.*, 2014). Though developing resistance in plants through conventional methods to protect them against viruses is expensive, it often takes years to breed resistance into desirable varieties. In order to sustain the agriculture production and maintain the quality produces, it demanded to use the biotechnological tools as one of the substantial techniques for the buildout of virus resistance in the plant to suppress the virus infection. The pioneering works on coat protein gene of *Tobacco mosaic virus*-mediated transgenic approach by Beachy (1986) opened up the perception of pathogen-derived resistance. Later, advancements in genetic engineering and plant transformation increased the probability of developing multiple plant virus control strategies. There are two main approaches in engineering virus resistance in plants. The genes can be originated from the pathogenic virus itself (pathogen-derived resistance) or from any other source (non-pathogen-derived resistance) (Dasgupta *et al.*, 2003). In pathogen-derived resistance (PDR), a portion or full viral genome is inserted into a plant; thereafter, it regulates or interferes with vital steps in the life cycle of the pathogen, which led to resistance in plants against virus infection. Diverse viral pathogen-

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derived genes such as coat protein, movement protein and replicase genes have been effectively utilized to buildout the virus-resistant transformants (Baulcombe, 2002; Senthilraja *et al.*, 2018; Gogoi *et al.*, 2019).

Importance of viral diseases on crop plants and their management

The proper management of virus disease is fundamental to farmers and agricultural industries to meet the demands. The virus diseases in several crops cause huge losses in terms of quantity and quality of agriculture produces. For example, the genus *Tospovirus* is one of the key viral pathogens of plants found to be economically important by threatening vegetable production in India. *Groundnut bud necrosis virus*, a species of *Tospovirus* causes nearly 80 per cent crop loss in tomato and other Solanaceous crops under field conditions (Kunkalikar *et al.*, 2010, 2011; Sushmitha and Bhat, 2014). Similarly, strains of *Cassava mosaic virus* cause more than 25 million tons of losses every year in Africa, India and Sri Lanka (Legg and Thresh, 2000). *Tungro virus* created epidemics during 2001 in West Bengal which resulted in loss of around 2911 million rupees (Muralidharan *et al.*, 2003). The economic loss in rice due to virus diseases from South East Asia was estimated around more than \$1.5 billion (Abo and Sy, 1998; Sasaya *et al.*, 2013). *Sri Lankan cassava mosaic virus* (SLCMV) belongs to the genus *Begomovirus* was found to be a severe pathogen of cassava causing mosaic disease in India and 84% yield losses has been documented in India (Thottapilly *et al.*, 2003). *Tobacco streak virus* (TSV), which belongs to the genus *Ilarvirus* (Family, *Bromoviridae*), has an extensive host range which infects several vegetable crops causing heavy crop loss (Jain *et al.*, 2005, 2008; Sivaprasad *et al.*, 2010). In addition to vegetable crops, it infects groundnut (Reddy *et al.*, 2002), sunflower (Ramiah *et al.*, 2001), soybean (Arun Kumar *et al.*, 2008), blackgram (Ladhalakshmi *et al.*, 2006) and cotton (Jagtap *et al.*, 2012; Vinodkumar *et al.*, 2017) in India. Besides, economic loss in terms of yield is under estimated from the field in several crops due to virus infection because it requires precise diagnostic techniques to determine the damage caused due to pathogen. Recent trends in food security and intensive agriculture have fetched new tasks to fight against virus diseases. Disease control by means of curative measures *i.e.* adaptation of cultural practices, is impossible due to variability in genome of virus and obligate nature of pathogen. It is necessary to control the disease before establishing pathogen in crops using different approaches called a prophylactic measure. In this context, several researchers have identified the beneficial microbe to induce host plant resistance against virus infection in several crops. In addition, identification of new

genes for developing the resistant plants through breeding programmes consumes more time and little laborious. It is necessary to apply advanced molecular biotechnological tools to develop genetically superior cultivars to suppress the virus disease in crop plants.

Sources of gene to engineer the plants for virus resistance

The rapid development in molecular biology techniques led to cloning and investigation of the genomic components of a variety of plant viruses. There are different strategies for transgenic resistance to plant virus.

1. Pathogen-derived resistance with the incorporation of pathogen components to the plant genome that interferes with the usual life cycle of the virus.
2. Pathogen-targeted resistance, relating to the inclusion of elements directly targeting viral genes and their products to make them non-functional.
3. Incorporation of prevailing genes from resistance plants into non-resistance plants.

Depending on the source of gene used, there are primarily two approaches for constructing genetically engineered resistance. The genes may be derived from the pathogenic virus itself (pathogen-derived resistance-PDR) or any other source. Non-pathogen-derived resistance involves using genes responsible for host tolerance and other genes regulating the adaptive host processes; genes provoked in response to pathogen attack to develop transgenics resistant to plant virus (Dasgupta *et al.*, 2003). The approaches for PDR are classified into two groups, those that require the development of proteins (e.g., coat protein, replicase, movement protein-mediated resistance) and those that need only the viral nucleic acid accumulation (e.g., dsRNA-mediated resistance, sense, antisense, and ribozyme) (Kalantidis *et al.*, 2002). In general, the earlier technique confer resistance to wide array of viruses, while the later provides high levels of resistance to a particular strain of virus. Several reports have been documented for the development of pathogen-derived virus resistance in plants. Enhancing the antiviral activity in plant species through gene silencing has proved to be effective in several plant-virus pathosystems.

Coat protein-mediated resistance (CPMR)

CPMR was first reported for *Tobacco mosaic virus* (TMV) in tobacco model system in 1986, and it has also been used to develop resistance to many viruses in various crop plants. CPMR may offer a broad range or narrow protection

to transgenic plants. The TMC coat protein (CP) provides the resistance to closely associated strains of TMV effectively and fading levels of resistance to tobamoviruses which share low level of sequence similarities with CP gene was noticed (Nejidat and Beachy, 1990). The transgenic lines of *Nicotiana benthamiana* developed using CP of *Cowpea aphid-borne mosaic virus* (CABMV) exhibited the delayed symptom development during the early stages of disease development (Mundembe *et al.*, 2009). Raj *et al.* (2005) showed that transgenic tomato cv. Pusa Ruby developed using the CP gene of *Tomato leaf curl virus* (TLCV) exhibited variable degrees of resistance or tolerance when plants were challenge inoculated with viruliferous whiteflies carrying the TLCV. Later, the plants were found without symptoms even after 75 days of challenge inoculation. Further, PCR analysis using replicase gene-specific primers produced negative results in plants challenge inoculated and suggested that they did not support accumulation and replication of virus, indicating the CP gene (TLCV- CP) arbitrated the resistance in plants. Saker (2003) produced transgenic potato lines harbouring the CP gene of *Potato virus Y* (CP-PVY), and transgenic potato lines expressing CP-PVY gene, confers resistance against PVY.

Movement protein-mediated resistance (MPMR)

The cell-to-cell movement of virus in a plants system is mediated by movement proteins (MP). It has been shown that movement proteins change the gating mechanism of plasmodesmata, allowing the particle of virus and their derivatives to spread to neighboring cells. However, pathogen-mediated resistance may also be engineered with dominant-negative mutant forms of viral genes. The usefulness of this strategy was demonstrated by the expression of viral MP in transgenic plants, that conferred resistance only when the transgene defined a dysfunctional MP. This phenomenon was first demonstrated in tobacco plants by producing modified MP to develop resistance against TMV, which is moderately active as a transgene. It is assumed that conferred resistance is based on the rivalry between the encoded MP gene of the wild-type virus and the preformed dysfunctional MP to bind to the plasmodesmatal sites (Lapidot *et al.*, 1993). In addition, the above resistance was shown to be successful against distantly related or dissimilar viruses (Lapidot *et al.*, 1993; Malyshenko *et al.*, 1993). The transgenic *N. benthamiana* plants expressing the modified 13K MP, determined by the central Triple Gene Block (TGB) ORF of *White clover mosaic potexvirus* (WCIMV), showed systemic resistance to WCIMV infection as well as expressed the resistance to *Potato virus S* (PVS) and two *Potexviruses* (Beck *et al.*, 1994). Cooper *et al.* (1995) reported that transgenic *N. tabacum* cv. Xanthi NN

lines express a gene encoding a dysfunctional MP (dMP+) of TMV that was developed with deletion of 3, 4 and 5 N-proximal amino acids, which are known to be immune to many *Tobamoviruses*. The sign of disease and systemic spread of virus in the upper leaves of challenge inoculated plants were delayed the symptom expression and reduced the accumulation of virus as systemic infection in the plants challenge inoculated with *Alfalfa mosaic alfamovirus*, *Cucumber mosaic virus*, *Tobacco ringspot virus*, *Tobacco rattle virus* and *Peanut chlorotic streak virus*.

Replicase protein-mediated resistance (Rep-MR)

The replicase proteins conferred the immunity to infection by the virus, usually restricted to the strain of the virus, from which the target gene was derived. It was first demonstrated in transgenic plants containing a sequence that codes a replicase fragment of 54 kDa against TMV (Golemboski *et al.*, 1990). A truncated replicase gene derived from *Cucumber mosaic virus* (CMV) subgroup I has deliberated maximum level of resistance in transgenic tobacco against subgroup I of CMV strains. However, transgenes never showed any resistance to other subgroup II or other viruses (Zaitlin *et al.*, 1994). Similarly, mutated replication-associated protein from *Maize streak virus* (MSV) used for maize transformation showed stable expression and plants showed a substantial decrease in symptom severity (Shepherd *et al.*, 2007). The mechanisms behind the suppression of virus was strongly believed to block the replication of virus and the protein formed by the transgene is proposed to interfere in some way with the replicase formed by the virus (Hellwald and Palukaitis, 1995; Heinlein, 2015; Lee *et al.*, 2019).

RNA silencing

The RNAi-based strategies have been explored as a supremacy tool to engineer the plant against plant viruses. The resistance attained in transgenic plants mainly due to post-transcriptional gene silencing induced by transgene via dsRNA formation is termed as RNA silencing (Baulcombe, 2002). RNA silencing has augmented the search of plant antiviral mechanisms that enable down-regulation of gene expression with maximum accuracy without affecting the expression of other genes. The resistance targets precisely the RNA genome and it has been referred to as RNA mediated resistance / RNA interference (RNAi) (Wang *et al.*, 2000; Grishok *et al.*, 2000; Hammond *et al.*, 2000; Smith *et al.*, 2000). RNA interference mechanism was first demonstrated in plants, where insertion of additional copies of flower pigmentation responsible chalcone synthase gene resulted in the suppression of sequence-specific target and the endogenous RNA. Subsequently,

transgenic lines were developed to produce reduced pigmentation in flowers or even the complete absence of pigmentation (Napoli *et al.*, 1990; Van der Krol *et al.*, 1990). RNAi can be induced in plants by delivering expression vectors similar to self-complementary dsRNA (Horiguchi, 2004; Watson *et al.*, 2005) (Figure 1). Some experimental studies have revealed that inverted repeat constructs that encode hairpin RNAs (self-complementary RNAs) can induce RNA silencing effectively in plants and leads to high frequencies of resistance in transgenic lines (Chen *et al.*, 2004). Helliwell and Waterhouse (2003) described that RNAi constructs have a spacer sequence between an inverted repeat and the resulting transcript from stem-loop structure from the RNAi construct and these transcripts are often called hairpin RNAs (hpRNAs). The increased gene silencing effect of constructs was attained by the existence of an intron in between the two complementary regions (Smith *et al.*, 2000). They also demonstrated that plants transformed with a construct comprising sense and antisense sequences of Nla protease (*Pro*) gene of *Potato virus Y* with flanking with the spacer fragment size of 800-nucleotide derived from the *uidaA* (*GUS*) gene expressed the high level of resistance to virus and conferred the stability of the seamless inverted-repeat sequences.

RNA silencing in plants can serve as defense mechanism against virus infection and transposons (Vance and Vaucheret, 2001; Voinnet, 2001). It has been shown that the expression of inverted repeats in transgenic plants improved the resistance against the virus, suggesting the role for double-stranded (ds) RNA. Since dsRNA is not a normal constituent of the eukaryotic cells and it became evident that they are the key regulator in the process of gene silencing mechanism, which leads to degradation of RNAs that are homologous in transgene (Vaucheret and Fagard, 2001). The DNA stability was formed by eliminating the loop region of hpRNA by replacing the spacer with an intron sequence, but was spliced out during pre-mRNA processing (Smith *et al.*, 2000). The efficient silencing effect was documented in transformed plants expressing the inverted repeat constructs encompassing sense/anti-sense arms ranging from 98 to 853 nucleotide and inclusion of a spacer sequence (intron) in the constructs (Wesley *et al.*, 2001). Tougou *et al.* (2006) also demonstrated the effect of inverted repeat construct containing CP gene of *Soybean dwarf virus* (SbDV) spaced by β -glucuronidase (*GUS*) sequences. The resultant transgenic soybean lines inoculated with SbDV remained symptomless, suggesting the involvement of RNA silencing in the resistance. In animals, delivery of sense or antisense RNA led to endogenous messenger RNA degradation (Guo and Kempfues, 1995). This type of revolution in

animals was detected by the injection of dsRNA in *Caenorhabditis elegans* that resulted in endogenous mRNA degradation and it has been referred to as 'RNA interference' (RNAi) (Fire *et al.*, 1998). Subsequently, the discovery of shorter forms of small RNA (siRNA) i.e. ~25 nucleotides (nt), from the longer dsRNA has been considered as hall mark of RNA silencing (Hamilton and Baulcombe, 1999).

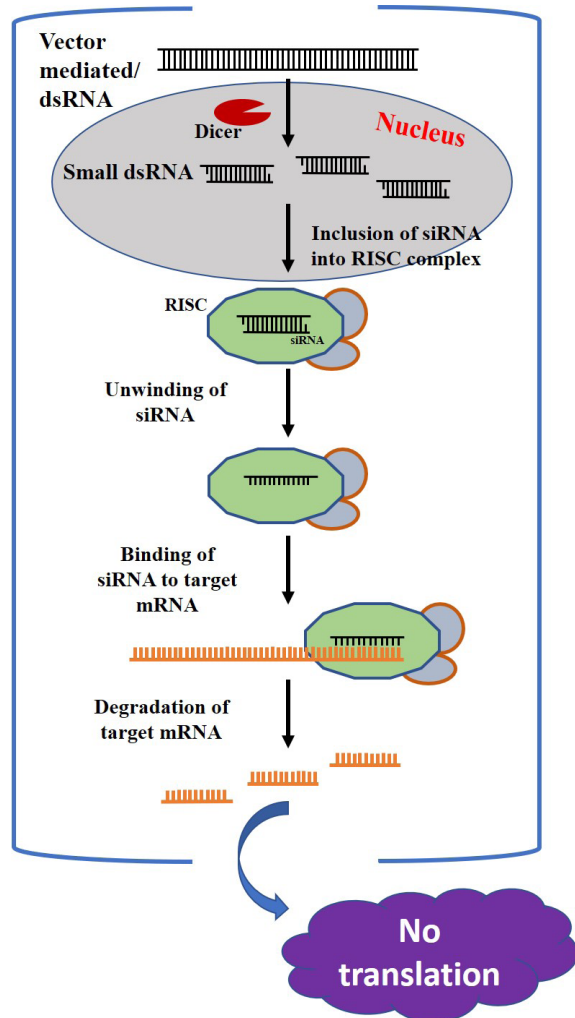


Figure 1. Schematic representation of RNAi mechanism in plants

Coat Protein (CP)- based RNAi

Pradeep *et al.* (2012) established that sunflower and tobacco transgenic lines that are expressed with inverted repeat-TSV CP gene showed the resistance to infection of TSV and lower levels of accumulation of TSV were observed compared with non-transgenic plants. Guo *et al.* (2015) developed the transgenic sugarcane expressing CP gene of *Sorghum mosaic virus* (SrMV) based on the RNA silencing approach. It was reported that transgenic line expressing the RNA cassettes showed resistance against SrMV upon artificial inoculation. Similarly, tobacco plants transformed with hpRNA containing CP gene of TSV through the *Agrobacterium*, mediated

Table 1. RNAi mediated resistance in plants against virus diseases

Crop	Virus	Gene used for resistance	Reference
Tobacco / rice	<i>Potato virus Y</i> (PVY)	Protease (Pro) gene	Waterhouse <i>et al.</i> (1998)
Tobacco	<i>Cotton leaf curl virus</i> (CLCuV)	AC1 double-stranded RNA	Asad <i>et al.</i> (2003)
<i>Arabidopsis thaliana</i>	<i>Turnip mosaic virus</i> (TuMV)	Coat protein	Nomura <i>et al.</i> (2004)
Tomato	<i>Tomato yellow leaf curl virus</i> (TYLCV)	AC1 double-stranded RNA	Yang <i>et al.</i> (2004)
Papaya	<i>Papaya ringspot virus W</i> (PRSV-W)	Coat protein	Krubphachaya <i>et al.</i> (2007)
Soybean	<i>Soybean dwarf virus</i> (SbDV)	Coat protein	Tougou <i>et al.</i> (2006)
Tobacco	<i>Cucumber green mottle mosaic virus</i> (CGMMV)	Coat protein	Kamachi <i>et al.</i> (2007)
Groundnut	<i>Tobacco streak virus</i> (TSV)	Coat protein	Bag <i>et al.</i> (2007)
Tomato	<i>Tomato leaf curl virus</i> (ToLCV)	Replicase	Ramesh <i>et al.</i> (2007)
Common Bean	<i>Bean golden mosaic virus</i> (BGMV)	Replicase	Bonfim <i>et al.</i> (2007)
Rice	<i>Rice tungro bacilliform virus</i> (RTBV)	DNA encoding ORF IV	Tyagi <i>et al.</i> (2008)
Tobacco	<i>Potato virus Y</i> (PVY)	Coat protein	Vargas <i>et al.</i> (2008)
Lettuce	<i>Mirafiori lettuce virus</i> (MiLV)	Coat protein	Kawazu <i>et al.</i> (2009)
Cassava	<i>Cassava brown streak Uganda virus</i> (CBSUV)	Coat protein	Yadav <i>et al.</i> (2011)
Tobacco	<i>Tobacco streak virus</i> (TSV)	Coat protein	Pradeep <i>et al.</i> (2012)
Banana	<i>Banana bunchy top virus</i> (BBTV)	Replicase	Elayabalan <i>et al.</i> (2013)
Tomato	<i>Cucumber mosaic virus</i> (CMV)	Replicase	Ntui <i>et al.</i> (2014)
Tobacco	<i>Tobacco streak virus</i> (TSV)	Coat protein	Rajamanickam <i>et al.</i> (2015a)
Tobacco	<i>Tobacco streak virus</i> (TSV)	Replicase	Rajamanickam <i>et al.</i> (2015b)
Rice	<i>Rice stripe virus</i> (RSV)	Coat protein	Li <i>et al.</i> (2016)
Banana	<i>Banana bunchy top virus</i> (BBTV)	Replicase	Elayabalan <i>et al.</i> (2017)
Groundnut	<i>Tobacco streak virus</i> (TSV)	Coat protein	Senthilraja <i>et al.</i> (2018)
Cassava	<i>South African cassava mosaic virus</i> (SACMV)	Replicase	Walsh <i>et al.</i> (2019)
Tobacco	<i>Sri Lankan cassava mosaic virus</i> (SLCMV)	Coat protein	Gogoi <i>et al.</i> (2019)
Tomato	<i>Groundnut bud necrosis virus</i> (GBNV)	Coat protein, Replicase protein	Suganyadevi (2019)

transformation exhibited resistance to TSV upon mechanical inoculation and ELISA confirmed the lower level of virus titre in transgenic tobacco lines (Rajamanickam *et al.*, 2015a). Gogoi *et al.* (2019) found that transgenic tobacco plant expressing sense and antisense orientation of CP gene of *Sri Lankan cassava mosaic virus* (SLCMV) showed resistance to SLCMV. The Northern blot analysis of tobacco line confirmed the expression of virus specific siRNAs, indicated that RNA silencing technology has been adopted in virus resistance.

Jia *et al.* (2017) developed the transgenic lines that are expressed with CP gene of *Papaya ringspot virus* (PRSV) showed a wide spectrum of resistance against PRSV I, II and III subgroup and Northern blot analysis the production of siRNA in transgenic lines confirms the RNA interference. Senthilraja *et al.* (2018) investigated the development of transgenic peanuts by expressing the hpRNA cassettes containing CP gene of TSV through *Agrobacterium*-mediated transformation. The developed transgenic line showed resistance against TSV upon sap inoculation

under greenhouse conditions, which suggested that the resistance in peanut plants against TSV was induced through genetic manipulation by expressing dsRNA of CP gene of TSV as marker for RNAi. Chen *et al.* (2019) found that chimeric hpRNA comprising CP genes of *Cymbidium mosaic virus* (CymMV) and *Odontoglossum ringspot virus* (ORSV) expressed in tobacco showed normal growth and produced no symptoms when inoculated with CymMV and ORSV as mixed inoculum, which conferred the RNA mediated interference.

Movement Protein (MP) based RNAi

The MP gene of several viruses has been demonstrated to be the useful gene to suppress the disease in transgenic plants. For example, RNA interference-based gene construct containing part of movement protein gene derived from *Chickpea chlorotic dwarf Pakistan virus* (CpCDPKV) expressed in *N. benthamiana* showed the optimistic resistance against CpCDPKV (Nahid *et al.*, 2011). Similarly, transgenic tobacco lines expressed with hpRNA comprising MP gene of *Tobacco mosaic virus* (TMV) exhibited comprehensive resistance to TMV. Furthermost, high level of TMV replication was observed in all the tobacco control plants, whereas an undetectable level of TMV multiplication was observed in transgenic plants challenged with CMV and TMV (Hu *et al.*, 2011). The artificial miRNA169a consisting of 21 nucleotides derived from V2 gene (movement protein) region of *Cotton leaf curl Burewala virus* (CLCuBuV) transformed in cotton showed partial resistance against CLCuBuV and artificial inoculation of transgenic lines showed complete resistance against *Cotton leaf curl Kokhran virus* (CLCuKoV).

Further analysis revealed the possibility of RNA-mediated resistance in transgenic cotton (Ali *et al.*, 2013). Ntui *et al.* (2015) revealed that cassava-transgenic lines expressing the dsRNA derived from part of AV2 and AV1 (movement protein) of *Sri Lankan cassava mosaic virus* (SLCMV) showed high level of resistance against SLCMV. The Northern blot analysis confirmed the presence of siRNA specific to target gene of SLCMV, indicated the RNA silencing mediated resistance. Similarly, double-strand RNA derived from movement protein gene of *Sesbania mosaic virus* (SeMV) showed resistance in *Sesbania* plants against SeMV and MP gene-derived dsRNA provided higher level of resistance compared to dsRNA derived from CP gene. The sequence specific target conferred the resistance mechanisms in *Sesbania*, which indicated the RNA silencing mediated resistance (Konakalla *et al.*, 2019).

Replicase based RNAi

The transgenic tobacco plants expressed with hpRNA containing the part of replicase gene of

Chickpea chlorotic dwarf Pakistan virus (CpCDPKV) impaired the symptom expression of CpCDPKV and real-time PCR analysis confirmed the lower level of virus titre in transgenic lines (Nahid *et al.*, 2011). Shekhawat *et al.* (2012) developed the transgenic banana expressing the intron-hpRNA comprised with replication initiation protein (Rep) of *Banana bunchy top virus* (BBTV). The transgenic lines were resistant to BBTV and siRNA analysis confirms the mechanism based on RNA interference. Elayabalan *et al.* (2013) developed transgenic banana plants expressing the replicase gene of *banana bunchy top virus* (BBTV) through *Agrobacterium*-mediated transformation. Transgenic lines expressing RNAi-rep gene construct showed resistance to BBTV; symptomless and suppressed level of replication of virus was also observed. Jada *et al.* (2014) found that full-length expression of TMV in tobacco plants showed resistance against TMV and expressed the multiple defense mechanisms, including the presence of reduced level of viral replication in virus challenged transgenic lines. Peng *et al.* (2014) found that transgenic tobacco harboring hpRNA derived from RNA dependent RNA polymerase of *Watermelon silver mottle virus* (WSMoV) exhibited the broad-spectrum resistance against different *Tospoviruses* like *Groundnut yellow spot virus*, *Groundnut chlorotic fan spot virus*, *Impatiens necrotic spot virus* and *Tomato spotted wilt virus*.

Similarly, transgenic tomato expressing the same construct showed resistance against WSMoV and other *Tospoviruses*. The tobacco plants transformed with hpRNA containing replicase gene of TSV through the transformation mediated by *Agrobacterium* exhibited resistance to TSV upon mechanical inoculation and ELISA confirmed the lower level of virus titre in transgenic tobacco lines (Rajamanickam *et al.*, 2015b). The squash plants expressing the hpRNA construct derived from replicase gene of *Squash leaf curl virus* (SqLCV) showed resistance against SqLCV. Further, qPCR analysis confirmed reduction in virus accumulation of virus genome in transgenic plants (Taha *et al.*, 2016). Elayabalan *et al.* (2017) developed the RNAi gene construct corresponding to replicase gene of *banana bunchy top virus* (BBTV). The banana cv. Virupakshi (AAB) plants injected with *Agrobacterium*-containing construct did not produce any symptoms of BBTV after 45 days of infection whereas, non-injected plants have produced the symptoms, suggested the RNA mediated resistance. Suganyadevi *et al.* (2019) developed the hpRNA construct corresponding to replicase gene of *groundnut bud necrosis virus* (GBNV). The tomato cv. PKM1 injected with *Agrobacterium* containing construct through *in-planta* transformation technique did not produce any symptoms, whereas non-transgenic plants produced the symptoms.

Table 2. List of viral proteins act as suppressors

S. No	Virus	Genus	Genome	Viral proteins	Mechanism of action	Reference
1.	<i>Tobacco streak virus</i>	Illavirus	RNA	2b	-	Valentine <i>et al.</i> (2004)
2.	<i>Cucumber Mosaic Virus</i>	Cucumovirus	RNA	2b	-	Duan <i>et al.</i> (2012)
3.	<i>Citrus leaf blotch virus</i>	Citricivirus	RNA	MP	-	Renovell <i>et al.</i> (2012)
4.	<i>Tomato spotted wilt virus</i>	Tospovirus	RNA	NSs	Sequesters both long dsRNA and double-stranded siRNA or miRNA	Schnettler <i>et al.</i> (2010)
5.	<i>Beet yellows virus</i>	Closterovirus	RNA	P21	Sequesters siRNA duplexes	Ye and Patel (2005)
6.	<i>Citrus tristeza virus</i>		RNA	p23, capsid protein, p20	-	Lu <i>et al.</i> (2004)
7.	<i>Sweet potato chlorotic stunt virus, Tomato chlorosis virus</i>	Crinivirus	RNA	P22	Sequesters siRNA duplexes	Kataya <i>et al.</i> (2009)
8.	<i>Beet western yellow virus</i>	Polerovirus	RNA	PO protein	Targets AGO1 to degradation	Csorba <i>et al.</i> (2010)
9.	<i>Pea enation mosaic virus</i>	Tritimovirus	RNA	PO protein	Targets AGO1 to degradation	Fusaro <i>et al.</i> (2012)
10.	<i>Wheat streak mosaic virus</i>		RNA	P1 Protein	-	Young <i>et al.</i> (2012)
11.	<i>Rice stripe virus</i>	Tenuivirus	RNA	NS3	Sequesters siRNA duplexes	-
12.	<i>Red clover necrotic virus</i>	Dianthovirus	RNA	MP	-	
13.	<i>Turnip yellow mosaic virus</i>	Tymovirus	RNA	P69	-	Chen <i>et al.</i> (2004)
14.	<i>Tobacco mosaic virus</i>	Tobamovirus	RNA	Small replicase subunit		Vogler <i>et al.</i> (2007)
15.	<i>Tomato yellow leaf curl virus</i>	Begomovirus	DNA	C4	Sequesters siRNA duplexes	Luna <i>et al.</i> (2012)
16.	<i>Mungbean yellow mosaic virus</i>		DNA	TrAP / AC2	-	Trinks <i>et al.</i> (2005)
17.	<i>Banana bunchy top virus</i>	Babuvirus	DNA	Clink, MP	-	Niu <i>et al.</i> (2009)

Applications of RNAi

RNA interference is a useful reverse genetic technology for evaluating gene functions in a variety of species and organisms. It has been first adopted to determine the role of chromosome I and II of *Caenorhabditis elegans* through gene knockout techniques. The study identified the role of these genes involved in embryonic development and cell division in *C. elegans* (Fraser *et al.*, 2000; Gonczy *et al.*, 2000). Similarly, the production of decaffeinated coffee plants was developed by knocking down the theobromine synthase gene using hairpin constructs (Ogita *et al.*, 2003). In addition, RNAi is useful in multipurpose applications like disease control in crops, specifically the viruses, treatment of tumors in medical application, control of bacterial and parasites in the medical industry, drug development and production of model animal for research (Pereira and Cendes, 2013). RNAi can promote the screening and production of drugs by discovering genes that can impart drug resistance or genes whose mutant phenotypes are ameliorated by drug therapy, providing information on modes of

action of new compounds. It was also a preferred method of application to investigate the concurrent functions of multiple genes expressing the functions in organisms, where duplication occurs concerning a specific function, as many of these genes may be silenced simultaneously. The polymer and lipid-nanoparticles-based delivery of siRNA was effective in delivering the siRNA into the human body that facilitates the broad translational success of RNA interference (RNAi) technology in therapeutic applications (Pottash *et al.*, 2019).

The gene silencing effect was first detected in petunia plants through the delivery of exogenous transgenes to up-regulate the activity of the chalcone synthase gene, an enzyme involved in the development of particular pigments (Napoli *et al.*, 1990). The incorporation of transgene resulted in the production of variegation with complete loss of color. This might be due to the introduced homologous DNA sequence, which indicated the possible mechanism of suppression of endogenous loci and the phenomenon was called as "co-suppression" (Hannon, 2002). Later, the technology

was instrumental in agriculture for crop development and enhanced plant nutritional value (Abdolhamid *et al.*, 2010). The RNAi technology can also be used effectively to improve seed germination and nutritional quality in plants. A high-lysine maize variant has been developed by knocking out the expression of the 22-kD maize zein storage protein (Segal *et al.*, 2003). The *myo*-inositol-3-phosphate synthase (*MIPS1*) gene from soybean is involved in phytic acid (PA) biosynthesis. PA is the major phosphorus reserve material in soybean seeds and it is a potent iron chelator, causing deficiencies that lead to malnutrition. The disrupted *MIPS1* gene through RNAi approach, limiting the enzyme PA biosynthesis in soybean seeds, leads to improved quality of seed with more of minerals (Kumar *et al.*, 2019). RNA-induced silencing technology has become one of the favored methods for inducing resistance to viruses in plants. RNAi has been exploited for the management of several plant viruses in agriculture crops. Tobacco plant expressing coat protein and replicase genes of TSV showed resistance against TSV through RNAi mediated resistance mechanism (Rajamanickam *et al.*, 2015a,b). Cowpea plant employed with RNA silencing technology by expressing transcriptional activator protein AC2 (TrAP) has shown resistance against *Mungbean yellow mosaic India virus* (MYMIV) (Kumar *et al.*, 2017). The chimeric gene construct containing CP and silencing suppressor genes of *Cucumber mosaic virus* (CMV) and the *Potato virus Y* (PVY) expressed in the tobacco plant exhibited more siRNA than the construct containing a single hairpin structure. The transgenic tobacco plants showed resistance against mixed infection of CMV and PVY (Xie *et al.*, 2014). The genes from viral genomes used to develop transgenic plants to manage the virus diseases are appended in table 1.

Post-transcriptional gene silencing (PTGS)

Post-transcriptional gene silencing can be achieved either by degradation of transcripts or by translational inhibition by siRNAs. Most of the research carried out in *Arabidopsis thaliana* indicates signal as directed by small interfering RNAs (Xie *et al.*, 2004). In plants, RNA silencing pathways are greatly classified based on their functional requirements (Baulcombe, 2004; Meister and Tuschl, 2004). There are four pathways viz., micro RNA (miRNA) pathway, small interfering RNA pathway, RNA directed DNA methylation pathway and exogenic RNA silencing pathway. The concept of RNAi first came into the limelight of the world by the discovery of the role of purified dsRNA injected into the body of *Caenorhabditis elegans* (Fire *et al.*, 1998). After injection of dsRNA, which corresponds to the nucleotide segment of *unc22* encoded for a non-essential myofilament protein into an adult

nematode, corresponding decreased activity leads to severe twitching phenotype was noticed, dsRNA injected nematode showed weak twitching, where progenies were also strong twitchers. *Drosophila* embryo extracts has been well studied for the biochemistry of RNA silencing and recorded with a two-step reaction viz., initiation and effector phases. During the initiation step, longer dsRNA molecules are converted into siRNA or miRNA species. Small RNAs characteristic with the size of 21-26 bp contain 2 nt 3'-overhangs and 5'-phosphorylated termini formed by RNase-III-type enzymes from the Dicer protein family (Bernstein *et al.*, 2001). The molecules of siRNA and miRNA are distinct in their origin and structure, The miRNA molecules are host-encoded and derived from long single stranded (ss) RNAs (Bartel, 2004). During effector phase, one strand of the siRNA or miRNA duplex is loaded onto a ribonucleoprotein complex, the RNA induced silencing complex (RISC) for sequence specific identification of target RNAs (Schwarz *et al.*, 2003; Tomari and Zamore, 2005). Viruses can be the source, the target, or both the source and the target of silencing. Virus-mediated post-transcriptional gene silencing (PTGS) can occur with RNA viruses which can replicate in the cytoplasm and also for DNA, which replicate in the nucleus (English *et al.*, 1996). Generally, sense and antisense components of transgenes were separated by an intron to increase the efficiency of PTGS (Smith *et al.*, 2000). The component involved in the RNA silencing mechanism are dicer which belongs to the RNase III family, Argonaute2 protein, RNA and DNA helicases, translation initiation factor, RNA-dependent RNA polymerase and transmembrane protein as channel or receptor.

Enzymatic behavior of the members of the Argonaute (AGO) protein family assists the programmed RISC to divide complementary mRNAs or arrest their translation (Fagard and Vaucheret, 2000; Hammond *et al.*, 2000). In plants and animals, molecular and genetic research has discovered multiple pathways of RNA silencing where various small RNAs and RNA silencing proteins play a major role. The next big step in this silencing method is separating dsRNA into small dsRNA fragments, called dicer (Bernstein *et al.*, 2001). These short sequences of unique RNAs of 21-25 nucleotides are detectable in all cells where PTGS is involved (Hamilton and Baulcombe, 1999). The shorter dsRNAs are often referred to as "short interfering RNAs" because they constitute an intermediate that can trigger the silencing process. The siRNAs will be integrated into the RNA-inducing silence complex (RISC) induced with RNA (Hammond *et al.*, 2000), which is the actual sequence-specific endoribonuclease that cleaves single-stranded target RNA.

Table 3. List of viruses to develop VIGS vectors

Virus	Genus	Genome	Host	Sequence	Targeted gene	Reference
<i>Plum pox virus</i> (PPV)	<i>Potyvirus</i>	RNA	<i>Nicotiana benthamiana</i> <i>Prunus domestica</i>	Coat protein gene	Coat protein gene	Guo <i>et al.</i> (1999); Scorza <i>et al.</i> (2013)
<i>Papaya ring spot virus</i> (PRSV)	<i>Potyvirus</i>	RNA	<i>Papaya caricae</i>	Coat protein gene	Coat protein gene	Gonsalves <i>et al.</i> (2004)
<i>Potato leaf roll virus</i> (PLRV)	<i>Polerovirus</i>	RNA	<i>Solanum tuberosum</i>	Replicase protein gene	Replicase protein gene	Kaniewski and Thomas (2004)
<i>Potato virus Y</i> (PVY)	<i>Potyvirus</i>	RNA	<i>Solanum tuberosum</i> <i>Nicotiana benthamiana</i>	Coat protein gene GFP	Coat protein gene	Kaniewski and Thomas (2004) Jones <i>et al.</i> (1999)
<i>Cucumber mosaic virus</i> (CMV)	<i>Cucumovirus</i>	RNA	<i>Cururbita pepo</i> <i>Petunia hybrid</i> and <i>Solanum lycopersicum</i>	Coat protein gene 2b protein	Coat protein gene CHS-A gene for chalcone synthase	Tricoli <i>et al.</i> (1995) Kanazawa <i>et al.</i> (2010)
<i>Zucchini yellow mosaic virus</i> (ZYMV), <i>Watermelon Mosaic Virus</i> (WMV)	<i>Potyvirus</i>	RNA	<i>Cururbita pepo</i>	Coat protein gene	Coat protein gene	Tricoli <i>et al.</i> (1995)
<i>Tobacco etch virus</i> (TEV)	<i>Potyvirus</i>	RNA	<i>Nicotiana benthamiana</i>	Coat protein gene	Coat protein gene	Lindbo <i>et al.</i> (1993)
<i>Pea seed-borne mosaic virus</i> (PSbMV)	<i>Potyvirus</i>	RNA	<i>Pisum sativum</i>	Replicase protein gene	Replicase protein gene	Jones <i>et al.</i> (1998)
<i>Tobacco rattle virus</i> (TRV)	<i>Tobravirus</i>	RNA	<i>Nicotiana benthamiana</i> <i>Solanum lycopersicum</i>	NbPDS NbChIH sIPDS	Phytoene desaturase, Magnesium chelatase 1-deoxy-D-xylulose-5-phosphate synthase	Senthil Kumar and Mysore (2011)
<i>Barley stripe mosaic virus</i> (BSMV)	<i>Hordeivirus</i>	RNA	<i>Hordeum vulgare</i>	<i>PDS</i> gene	<i>Phytoene desaturase</i>	Bruun Rasmussen <i>et al.</i> (2007)
<i>Apple latent spherical virus</i> (ALSV)	<i>Cheravirus</i>	RNA	<i>Glycine max</i>	PDS	Phytoene desaturase	Yomagishi <i>et al.</i> (2011)
<i>Pea early browning virus</i>	<i>Tobravirus</i>	RNA	<i>Pisum sativum</i>	<i>pds, uni, kor</i>	<i>pds, uni, kor</i>	Constantin <i>et al.</i> (2004)
<i>Poplar mosaic virus</i>	<i>Carlavirus</i>	RNA	<i>N. benthamiana</i>	<i>gfp</i>	<i>gfp</i>	Naylor <i>et al.</i> (2005)
<i>Potato virus X</i>	<i>Potexvirus</i>	RNA	<i>A. thaliana</i> , <i>N. benthamiana</i>	<i>gus, pds, DWARF, SSU, NFL, LFY</i>	<i>gus, pds, DWARF, SSU, NFL, LFY</i>	Ruiz <i>et al.</i> (1998)
<i>Bean pod mottle virus</i>	<i>Cucumovirus</i>	RNA	<i>Glycine max</i>	<i>PDS</i>	Phytoene desaturase	Zhang <i>et al.</i> (2006)
<i>Brome mosaic virus</i> (BMV)	<i>Bromovirus</i>	RNA	<i>Hordeum vulgare, Oryza sativa</i> and <i>Zea mays</i>	<i>pds, actin 1, rubisco</i> activase	<i>pds, actin 1, rubisco</i> activase	Ding <i>et al.</i> (2006)
<i>Turnip yellow mosaic virus</i>	<i>Tymovirus</i>	RNA	<i>A. thaliana</i> <i>N. benthamiana</i>	<i>pds, lfy</i> <i>PDS</i>	<i>pds, lfy</i> Phytoene desaturase	Pflieger <i>et al.</i> (2008)
<i>African cassava mosaic virus</i> (ACMV)	<i>Begomovirus</i>	DNA	<i>Manihot esculenta</i> <i>N. tabacum</i>	<i>CYP79D2</i> Su	Phytoene desaturase Endogenous genes magnesium chelatase complex	Fofana <i>et al.</i> (2004)
<i>Cabbage leaf curl Virus</i>	<i>Begomovirus</i>	DNA	<i>Arabidopsis thaliana</i>	<i>gfp, CH42, pds</i>	<i>gfp, CH42, pds</i>	Turnage <i>et al.</i> (2002)
<i>Tomato golden mosaic virus</i>	<i>Begomovirus</i>	DNA	<i>N. benthamiana</i>	<i>su, luc</i>	<i>su, luc</i>	Peele <i>et al.</i> (2001)
<i>Tomato yellow leaf curl China virus</i> - associated b DNA satellite	<i>Begomovirus</i>	DNA	<i>N. benthamiana</i> <i>S. lycopersicum</i> , <i>N. glutinosa</i> , <i>N. tabacum</i>	<i>pcna, pds, su, gfp</i>	<i>pcna, pds, su, gfp</i>	Tao <i>et al.</i> (2004)
<i>Bean golden mosaic virus</i> (BGMV)	<i>Begomovirus</i>	DNA	<i>Phaseolus vulgaris</i>	Replicase gene	Replicase gene	Faria <i>et al.</i> (2016)

RNAi can be caused in plants by the application of expression vectors that transcribes a self-complementary dsRNA (Horiguchi, 2004; Watson *et al.*, 2005). Several studies have shown that inverted repeat construct encodes self-complementary RNAs (hairpin RNAs) and effectively induced RNA silencing, leading to high resistance in transgenic plants (Smith *et al.*, 2000; Chen *et al.*, 2004). In transgenic plants, gene silencing can be achieved with constructs that express self-complementary RNA containing sequences homologous to the target genes (Helliwell and Waterhouse, 2003). These RNAi constructs include a series of spacers between an inverted repeat and the corresponding transcript from the stem-loop structure of the RNAi construct. These transcripts are thus sometimes referred to as hairpin RNAs (hpRNAs). Spacer sequence in the vector allows stable replication of RNAi plasmids in *E. coli* (Lee and Carthew, 2003). pHANNIBAL and pKANNIBAL with bacterial ampicillin and kanamycin-resistant genes, respectively, were designed by Wesley *et al.* (2001). For the development of hairpinRNA (hpRNA), a loop of 30-50 bases depending on various restriction sites were used to insert targeting gene sequences. In addition, it has been reported that the hpRNA constructs containing sense/anti-sense arms range from 98 to 853 nucleotides gave efficient silencing in a wide range of plant species and inclusion of an intron in these constructs had a consistently enhancing effect. The degree of silencing was observed 90-100% greater than that obtained in either co-suppression or antisense constructs. The presence of intron in between two complementary regions enhances silencing efficiency (Smith *et al.*, 2000).

Suppression of gene silencing

RNA silencing is a conserved eukaryotic event in the eukaryotic pathway that involves clampdown of specific gene expression facilitated by 21-24nt siRNA. Previously, an antiviral function of RNA silencing in a transgenic plant after infection with a potyvirus was demonstrated through molecular analysis (Lindbo *et al.*, 1993). Antiviral function explained the accumulation of siRNAs which are derived during viral infection at high level. Symptoms were appeared in the infected plants, and later, plants were recovered from the infection and become resistant to ensuing infection with the same virus. More studies reported and supported this model (Table 2). To counteract on RNAi antiviral defense, viruses from all the genera encoded for viral suppressors of RNA silencing. It inhibits major steps of RNAi system. Viral suppressing pathways primarily inhibit the siRNA biogenesis by preventing the recognition of siRNA by the AGO effector, RNA-induced silencing complex, and enzymes responsible for DNA-methylation in the host.

Suppression of RNA silencing through viral suppressors

A) Inhibiting viral RNA sensing and dicing

Two viral proteins like P14 of *Pothos latent aureusvirus* and P38 of *Turnip crinkle virus* bind with dsRNA in a size-independent way (Merai *et al.*, 2005, 2006). It has been shown that P38 inhibits the DCL4 in the *Arabidopsis* (Deleris *et al.*, 2006). P38 protein interferes with AGO1 binding, which leads to inhibition of DCLS (Azevedo *et al.*, 2010). In *Cauliflower mosaic virus* (CaMV), P6 has been shown as viral translational trans-activator protein for virus-derived small interfering RNAs (vsiRNA). P6 protein was recorded as RNA silencing suppressor and it binds with dsRNA, which has been required for the functioning of DCL4 (Haas *et al.*, 2008).

B) Inhibiting the RNA induced silencing complex (RISC)

Viral suppressor of RNA silencing can prevent RISC complex. They can accomplish target AGO proteins, siRNAs and miRNAs in a wide variety of mechanisms. Among the various suppression activities, the most common strategy is dsRNA suppression, which stops the assembly of the RISC effector (Lakatos *et al.*, 2006; Csorba *et al.*, 2007, 2010; Wu *et al.*, 2010). The P19 protein is the best-known viral suppressor of RNA silencing (VSRs) in tobamoviruses, which binds with ds siRNA having high affinity (Vargason *et al.*, 2003). For all biogenesis processes of siRNAs and miRNAs, 2'-O methylation step is highly essential. Viral protein *viz.*, HC-Pro gene of *Tobacco etch virus*, P122/130 of *Tobamovirus* and P19 of *Carnation Italian ringspot virus* involves in preventing si/miRNA RISC assembly (Vogler *et al.*, 2007; Lozsa *et al.*, 2008; Yu *et al.*, 2006). During the suppression process by the VSRs, siRNA is sequestered and its binding is inactivated before incorporation into RISC complex resulted in the spread of viral infection in the plants.

C) Inhibiting the AGO protein

Assembly of RISC complex has been prevented directly or indirectly by interactions of RISC complex and VSRs (Burgyan and Havelda, 2011). In *Cucumber mosaic virus* (CMV), The 2b protein prevented the proliferation of long-range signal, which facilitates systemic infection of virus (Guo and Ding, 2002). Additionally, CMV 2b protein interacts with the PAZ domain and inhibits the slicing activity of AGO11 (Zhang *et al.*, 2006) and co-localized in the nucleus of the cell (Mayers *et al.*, 2000). *Tomato aspermy virus* (TAV), 2b protein binds with long dsRNA and inhibits the synthesis of viral secondary siRNAs (Diaz Pendon *et al.*, 2007). It is concluded that 2b protein has dual role in either by concealed siRNA or by relating with AGO1 and foiling the

assembly of RISC complex. Similarly, the P0 protein of polioviruses has also been involved in targeting the essential activities of RISC complex and AGO protein, resulted in suppression of RNA silencing through induced degradation (Baumberger *et al.*, 2007; Bortolamiol *et al.*, 2007).

Virus-induced gene silencing

Virus-induced gene silencing (VIGS) has been considered as a mechanism demonstrated with the homology-dependent degradation of RNA via plant-virus-derived vectors carrying endogenous gene sequences. Without the need for genetic transformation in plants, VIGS has been explored as an effective tool for reverse genetics and developed to evaluate the gene functions in various plant species. VIGS's success is due to five factors,

1. Methodology is simple and frequently involved with agroinfiltration or biolistic inoculation.
2. The results are attained within short period of time *i.e.*, some weeks after inoculation.
3. The tool avoids the transformation process and applicable to a greater or a wide variety of plants.
4. It shows the potential to silence several genes and
5. Can be used for forward and reverse genetics (Senthil Kumar and Mysore, 2011).
6. VIGS was first described as the mechanism of retrieval from virus diseased plants. Now the term VIGS is analogous to the process of knock-down expression of endogenous genes by a recombinant viral vector. VIGS is based on RNA interference (RNAi), which refers to gene expression interference mediated in a sequence-specific manner by tiny RNA. Pathway manifestations are referred to in plants as Post Transcriptional Gene Silencing (PTGS) (Lindbo *et al.*, 1993; Soosaar *et al.*, 2005), RNAi in animals (Fire *et al.*, 1998) and Quelling in fungi (Cogoni *et al.*, 1996). To develop the VIGS vectors, several RNA and DNA genomes have been used (Table 3) (Burch-Smith *et al.*, 2004). VIGS have been reported in both monocot like barley, wheat, rice, maize, ginger and banana and also in dicot plants like *Eschscholzia californica*, *Aquilegia vulgaris*, *Papaver somniferum* and *Thalictrum* species. Several viral vectors were obtained from positive-strand RNA viruses, which include TMV, *Potato virus X* and *Tobacco rattle virus* (Lange *et al.*, 2013) and DNA virus, like *African cassava mosaic virus* (ACMV) (Fofana *et al.*, 2004). For agro-inoculation, VIGS vectors were derived using a cDNA copy of the viral genome that is inserted into the binary vector and translated into the *A. tumefaciens* strain.

Mechanism of virus-induced gene silencing

VIGS is focused on the ability of plant viruses to activate a PTGS-related plant defense mechanism. Post-transcriptional gene silencing is activated by the perception of dsRNA. Both RNA and DNA viruses, during their genome expression or replication, a viral double-stranded RNA can be produced from various sources:

1. Transcriptions of DNA viruses cause RNA transcripts to overlap (Ding and Voinnet, 2007)
2. By cellular or viral RNA dependent RNA polymerase, viral ssRNA converted into dsRNA (Lu *et al.*, 2003)
3. Through intra-molecular base pairing, hairpin dsRNA (Molnar *et al.*, 2005).

RNA interference mechanism is a phenomenon in which small dsRNA evades the expression of targeted genes through the sequence-specific degradation of mRNA. VIGS is one of the approaches to studying the specific gene function in plants. RNA viruses produce dsRNA during the multiplication in the host by the action of virus-encoded RdRP. The TRV3 genome of *Tobacco rattle virus* modified to carry with target gene and agroinfiltration in three weeks old tobacco plants induced the silencing of sequence-specific target in newly emerging leaves confirms the VIGS mechanism (Senthil Kumar and Mysore, 2014). Similarly, VIGS target gene in sense and antisense orientations into the *Tobacco ringspot virus* by mutating the sequence following the CP stop codon for the transient expression of foreign genes. The VIGS of phytoene desaturase (PDS) in *N. benthamiana* lead to the development of noticeable photo-bleaching phenotypes (Zhao *et al.*, 2016). The viruses used to develop VIGS vectors is listed in Table 3.

Future research

The development of resistance in plants through conventional approaches is slow because of lack of knowledge on the genetic variation among plants against viruses. In this context, artificial double-stranded RNA (dsRNA) derived from the genome of virus offers a powerful way for resistance in host plants. RNAi-mediated silencing of viral genes such as replicase or coat protein leading to sequence-specific transcript degradation which can interfere with the viral replication process. The direct delivery of *in vitro*-transcribed dsRNA by mechanical inoculation interferes with the virus infection in a sequence specific manner. The dsRNA production is extremely low cost compared to regeneration of transgenic plants. This approach provides a reliable and potential tool for plant protection against virus diseases in future.

CONCLUSION

The RNA silencing technology is a powerful tool, which facilitates the wider applications in the field of agriculture to develop improved crop varieties and astounding plant with a wider biotic and abiotic stress resistance. The tool has well been exploited for the effective management of plant virus diseases. However, the exogenous application/delivery of RNAi methodology has to be standardized for effective suppression of plant viruses.

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