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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.

Keywords: *RNAi; Gene sources; Post-transcriptional gene silencing (PTGS); Silencing suppressors; VIGS*

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). S*ri 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* (WClMV), showed systemic resistance to WClMV infection as well as expressed the resistance to *Potato virus* S (PVS) and two *Potex viruses* (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 NIa 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 Kemphues, 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).

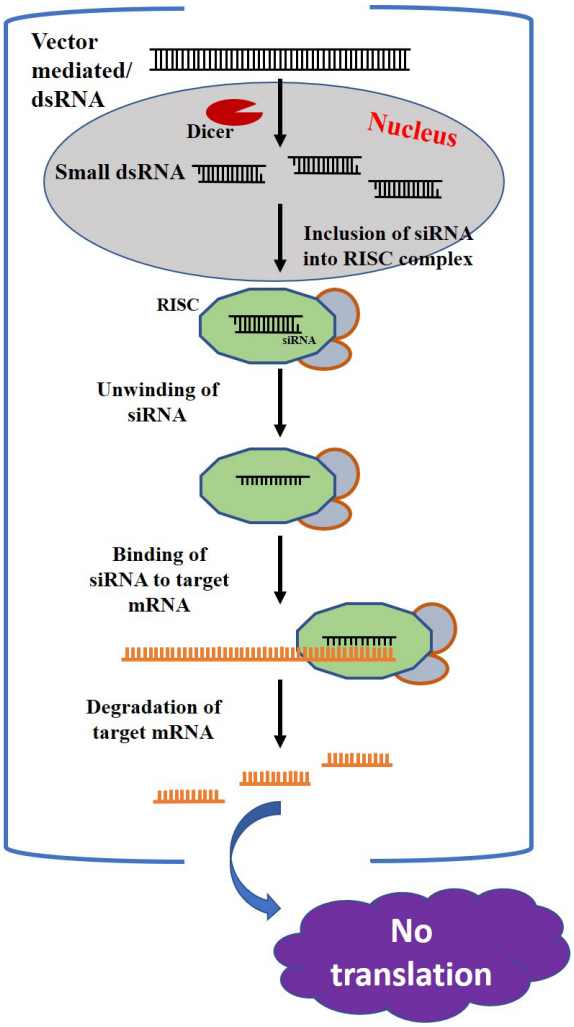

Figure 1. Schematic representation of RNAi mechanism in plants

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