# Overview of Acquired Virus Resistance in Transgenic Plants

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**Abstract**

Plant viruses have a strong negative impact on agricultural crops throughout the world. This fact makes the use of transgenic resistant plants as the only useful and potential virus control measures. All of the antiviral strategies used, till to date, were based on protective molecular immune mechanisms, evolved naturally in plants against viral diseases. This review is intended to provide the reader a framework of precise mechanisms involved behind the resistance strategies which offer suppression/blockage of viral infection in transgenic plants.

**Keywords:** RNAi, PTGS, RNAi suppressors, pathogen derived resistance, helper component proteinase

**Abbreviations:** RNAi—RNA interference; PTGS—Post Transcriptional Gene Silencing; PDR—Pathogen Derived Resistance; CP—Capsid Protein; MP—Movement protein; dsRNA—double stranded RNA; siRNA—small interfering RNA; RISC—RNA Induced Silencing Complex

## 1. Introduction

Up till now, more than 1200 plant viruses have been reported which include 250 of those viruses that cause significant losses in crop yield (Beachy, 1997). These, thus have a negative impact on agricultural crop production throughout the world. Plant pathologists and agronomists have made considerable efforts to control viral diseases which require an understanding of the virus replication, study about viral vectors and obviously, the deployment of useful genes for resistance in high-yielding varieties.

Before the dawn of genetic engineering, traditional plant breeding methodologies were sometimes successful in creating resistance to viruses in agronomically important crops. Initially, there have been a series of attempts whose basic aim was to generate resistance in plants against viruses. These attempts were based on Pathogen derived resistance where genes/genome fragments of virus origin were expressed (Baulcombe, 1994; Beachy, 1993; Lomonossoff, 1995; Wilson, 1993) and were met with mixed success. Since then, various viral sequences that encode structural and non-structural proteins were shown to confer resistance. Subsequently, small, double stranded, non-coding viral RNAs were shown to be of potential importance in transgenic plants for virus resistance, this led to the discovery of a novel innate resistance in plants, 'RNA silencing'.

RNAi/PTGS is a potential tool for rendering transgenic plants virus-resistant and involves the expression of a sequence homologous to the invading virus. In addition, although many plants may combat virus infections by gene silencing, there is a strong evidence that some of the plant viruses can fight back leading to suppression of the plant's ability to carry out the silencing process. These proteins are called as suppressors of this RNA silencing process (Brigneti *et al.* 1998; Voinnet *et al.* 1999). These suppressor proteins influence the final steady-state level of virus accumulation as strong suppressors would allow virus accumulation to be prolonged and at a high level. Conversely, if a virus accumulates at a low level it could be due to weak suppressor activity.

This review highlights the different strategies used to date to equip the plants with a defense machinery that enable them to protect themselves from a potential threatening group of pathogens, viruses. We aim to describe the proposed mechanisms behind the virus resistance that was acquired through various strategies.

## 2. Natural Defense of Plants against Viruses

Like other living organisms plants also have a natural defense mechanism (passive defense and active defense) against pathogens. Their passive defense is based on the presence of barriers such as rigid cell wall. Besides passive defense mechanism, plants also exhibit active defense which is triggered upon the recognition of the encountered pathogen. As the virus enters the cell, the plant responds either by hypersensitive response (HR) or extreme resistance (ER), during which the cells residing near the site of virus infection, rapidly commence to die (Goldbach *et al*. 2003). The natural defense of plants against invading pathogens is primarily due the presence of naturally produced secondary metabolites which are toxic in nature. The major compounds of secondary metabolites that defend plants are characterized as terpenes, phenolic compounds and nitrogen and sulfur containing compounds (Rosenthal *et al*. 1992; Van Etten *et al*. 2001).

Besides these secondary metabolites, plants also have resistance genes (R genes). Whenever a host plant is challenged by a pathogen (virus), the R genes are activated by recognizing the specific avirulence genes (avr) of the infecting virus (Keen 1990). This induces either the HR or ER response, leading to the ultimate death of the cells surrounding the site of infection. These responses are mediated by the increased production of ethylene, salicylic acid, jasmonic acid, nitric oxide, and many other nitrogen and sulphur containing compounds, (Mazid *et al*. 2011) followed by the activation of defence-related genes which include the genes that encode pathogenesis related (PR) proteins (Hammond-Kosack and Jones 1996; Lamb and Dixon 1997; Yang *et al*. 1997). In addition, local responses are accompanied by the development of systemic acquired resistance (SAR) in uninfected plant parts, which in turn enhance the resistance against challenging virus (Ryals *et al*. 1996; Sticher *et al*. 1997). Figure 1 outlines the mechanism of natural defense evolved in plants.

## 3. Cross Protection

Initially the virus resistance in plants was achieved by inoculating the plant with milder strain of the milder strain of target virus (Gadani *et al*. 1990). This type of protective measure is known as cross-protection. This technique was commonly employed on several important crops that include tomato, papaya and citrus (Beachy *et al*. 1990; Gadani *et al*. 1990; Hull and Davies 1992). Application of this technique met with mixed success. The main drawbacks of cross protection include; 1) degree of virulence of each viral strain vary from crop to crop, 2) milder strain of the virus that provides protection to one crop may cause serious diseases on varieties growing nearby. Therefore, it would be preferable to develop strategies that increase the degree of resistance. Figure 2 illustrate the antiviral strategies opted to-date in transgenic plants against target virus infection.

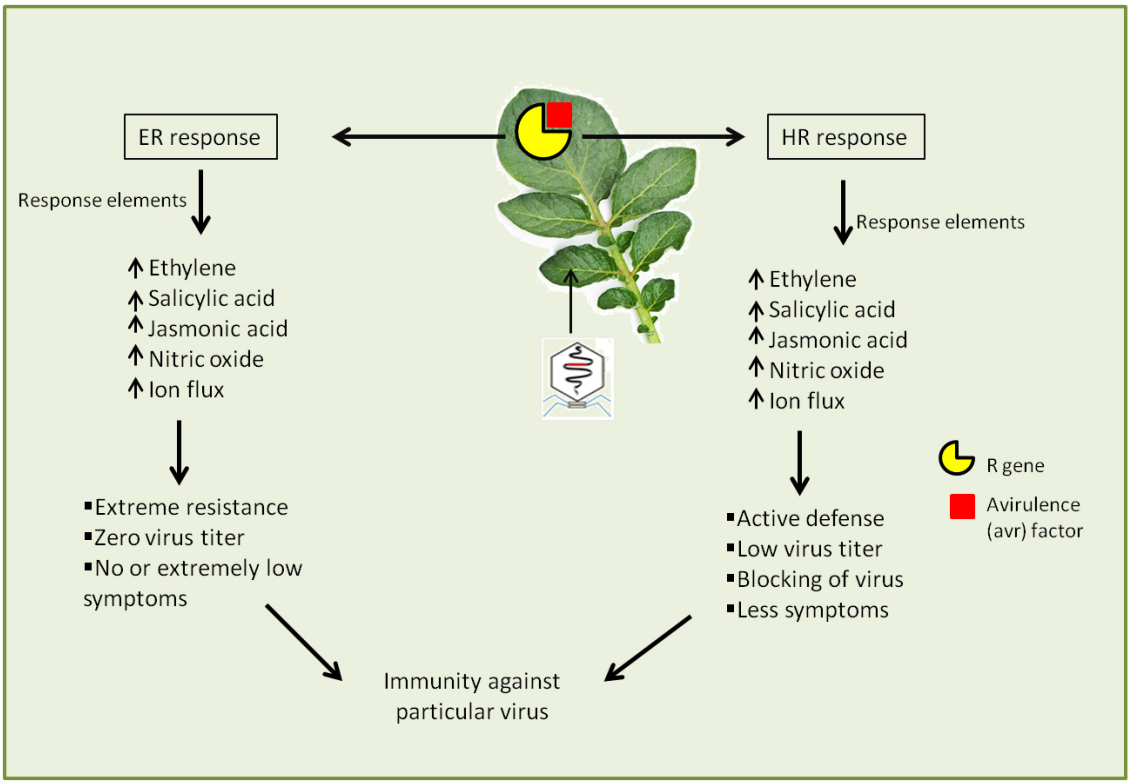


**Figure 1:** Natural defense in plants against viruses. Illustration of the Hypersensitive response (HR) and the Extreme Resistance (ER) mechanisms where production of secondary metabolites confers resistance against infecting virus

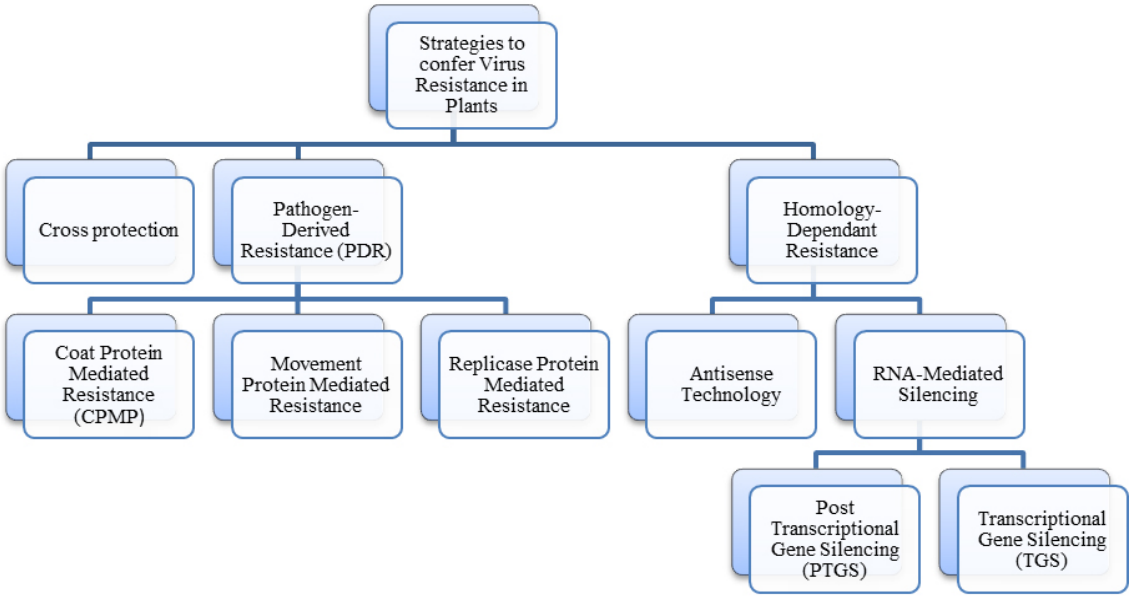


**Figure 2.** Schematic representation of the strategies opted to engineer virus resistant transgenic plants.

# 4. Pathogen Derived Resistance:

## 4.1 Definition

The concept of pathogen-derived resistance (PDR) strategy is based on the insertion of resistant genes that are derived from the pathogen (virus) into the host plant.

## 4.2 Strategies of Pathogen Derived Resistance:

Scientists have equipped the plants with gene based strategies to confer resistance against invasion of a certain pathogen including virus. Some of them require protein accumulation (coat protein mediated resistance, movement protein mediated resistance and replicase protein mediated resistance) for considerable resistance while others require accumulation of nucleic acid sequences (replicase mediated resistance).

## 4.3 Coat Protein Mediated Resistance:

In plant viruses, the major function of coat proteins (CPs) is disassembly of challenging virus accompanied by a later function in assembly of progeny virus (Tabassum *et al*. 2011). In addition CPs has a role in viral RNA translation, targeting the viral genome to its site of replication and severity of the infection.

In this strategy, coat protein gene is transformed in plants which ultimately form coat protein using host cell machinery. As the plant encounters the pathogen (virus), protein mediated response become visible. Possible mechanism behind this resistance include: 1) Coat protein produced from transgene is capable of subunit-subunit interaction (Clark *et al*. 1995) in which direct association of a small number (1 to 6) of transgene-derived CP molecules with the challenge virus during disassembly takes place. This interaction will ultimately prevent binding of ribosomes to the RNA of the invading virus, and hence infection, 2) Binding of coat protein to the host factors responsible for disassembly of the virion. This underlying mechanism will only be true for a plant containing a mutated transgene of coat protein. The mutated coat protein will offer a competitive inhibition to the coat protein of invading virus for binding to host factor involved in viral disassembly (Yusibov and Loesch-Fries 1995). Thus blocking the viral infection, 3) Coat protein may confer resistance against a specific virus by interacting with nuclear inclusion protein b (a replication protein), this possibility is specific for Potyviruses only (Hong *et al*. 1996).

## 4.4 Replicase-Mediated Resistance:

Plant viruses encode specific Replicase proteins that enable the virus to replicate in host cell. This event take place by co-interaction of virus encoded replicase protein and host protein factors. Plants having replicase transgene were reported to confer resistance against challenging inoculum.

Plants transformed with a modified RNA dependent RNA polymerase gene conferred resistance which was strain specific. Although protein was almost undetectable but if a mutant transgene encoding only 20% protein, result was ineffective resistance suggesting that protein was responsible for resistance (Carr *et al*. 1991; Golemboski *et al*. 1990). Possible mechanisms propose that the protein encoded by the transgene interferes with the function of the viral replicase, either by binding to viral proteins or host factors that regulate virus replication and its subsequent gene expression (Donson *et al*. 1993; Hellwald and Palukaitis, 1995). However, further experiments suggested the co-existence of both protein- and RNA-mediated resistance in plants expressing Replicase transgene (Goregaoker *et al*. 2000; Marano and Baulcombe, 1998; Tenllado *et al*. 1995, 1996). Replicase transgene trigger two possible mechanisms for resistance; one targeting replication of challenging virus in the cell (Carr *et al*. 1994; Hellwald and Palukaitis, 1995) while second limit the systemic spread of the virus by interacting with movement proteins (Nguyen *et al*. 1996; Wintermantel *et al*. 1997).

## 4.5 Movement Protein Mediated Resistance:

Plant viruses encode special movement proteins (MPs) which enable them to spread the infection between adjacent cells as well as systemically (Carrington *et al*. 1996). This movement involves

plasmodesmata and the channels that traverse plant cell walls and thus provide a systemic movement of virus between cells and tissues. Because several movement proteins were shown to accumulate in plasmodesmata, therefore the resistance rendered by movement proteins will strongly limit the viral infection.

A plant having transgenic expression of mutated viral movement protein will confer resistance through competition for plasmodesmatal binding sites between the mutant MP and functional MP of the challenging virus (Lapidot *et al*. 1993).

Whereas if the transgene specified a functional movement protein instead of dysfunctional one, it will neither affect virus infection nor have any effect on the rate of infection (Ziegler-Graff *et al*., 1991).

### 4.6 Antisense RNA Strategies:

Among Pathogen derived resistance strategies, antisense RNA (complementary to part of the viral genome) proves to have potential utility for protecting plants from systemic virus infection (Bejarano and Lichtenstein 1992). Antisense RNAs refer to small untranslatable RNA molecules that pair with a target RNA sequence on homology basis and thereby exert a negative control on interaction of target RNA with other nucleic acids or protein factors (figure 3). Further, RNase H cause an increase in rate of degradation of double stranded RNA (Culver 1995). This phenomenon completely operates on homology basis with target sequence.

Antisense RNA technology was quickly adapted by plant researchers because other approaches like homologous recombination and mutagenesis through gene-tagging used were based on reverse genetics and also these were not applicable in plants nor these were well developed. This background makes antisense RNA-mediated suppression more powerful tool for transgenic research and also for the development of commercial products (Chi-Ham *et al*. 2010).

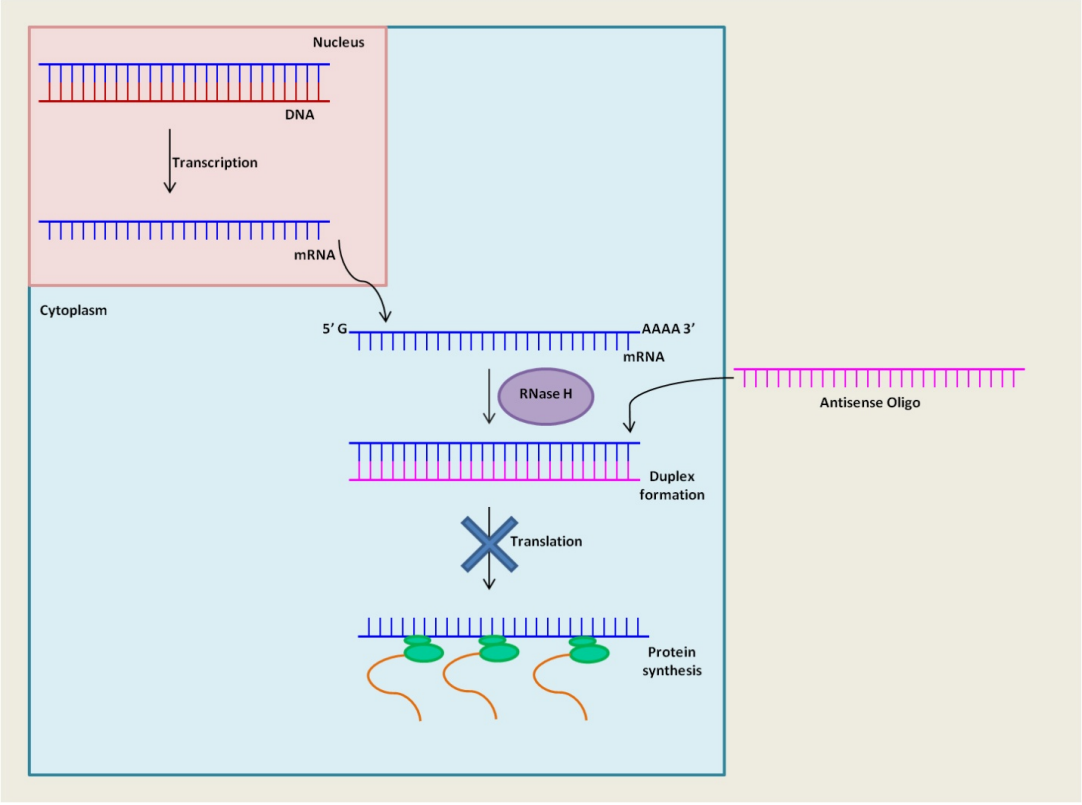


**Figure 3.** Mechanism of action of antisense oligonucleotide in suppressing the expression of target gene at transcriptional stage. RNase H is the endonuclease responsible for digestion of duplex RNA, thus blocking translation of target mRNA.

## 4.7 Post Transcriptional Gene Silencing (PTGS):

'RNA interference' is a conserved mechanism of Post transcriptional gene silencing (PTGS). It has rapidly gained favor as a "reverse genetics" tool to knock down the expression of targeted genes in plants. The term RNAi was coined in 1998 by Fire and Mello to describe a gene-silencing phenomenon based on double-stranded RNA (Fire *et al*. 1998). PTGS mechanism controls processes including development, the maintenance of genome stability and defense against molecular parasites (transposons and viruses). Several reports pointed out that PTGS in plants is strictly linked to RNA virus resistance mechanism (Joseph et al. 2012; Anandalakshmi *et al*. 1998; Brigneti *et al*. 1998; Kasschau and Carrington, 1998).

### *4.7.1 Mechanism of RNAi/PTGS:*

RNAi (RNA interference) is a natural defense pathway evolved in plants against viruses and potential transposons. It is a cellular pathway in which target sequences are degraded on homology basis at mRNA level by small RNAs, thereby preventing the translation of target RNAs. In plants, two functionally different RNAs; microRNA (miRNA) and small interferring RNA (siRNA), have been characterized. miRNAs are small 21-26nt long dsRNAs that are genome coded and are endogenous to every cell. Structurally, they comprised of a stem region which is double stranded and a loop region which is single stranded. miRNAs generated from endogenous hpRNA precursors and are basically involved in the regulation of gene expression during development (Bartel, 2004) and are reported to be strong inducers of RNA silencing when introduced as artificial miRNAs (Ossowski *et al*. 2008; Duan et al., 2012). On the other hand, siRNAs are generated from long dsRNA and are involved in defense through RNA interference (Lecellier and Voinnet, 2004; Vastenthouw and Plasterk, 2004). Figure 4 demonstrated mechanism of RNAi.

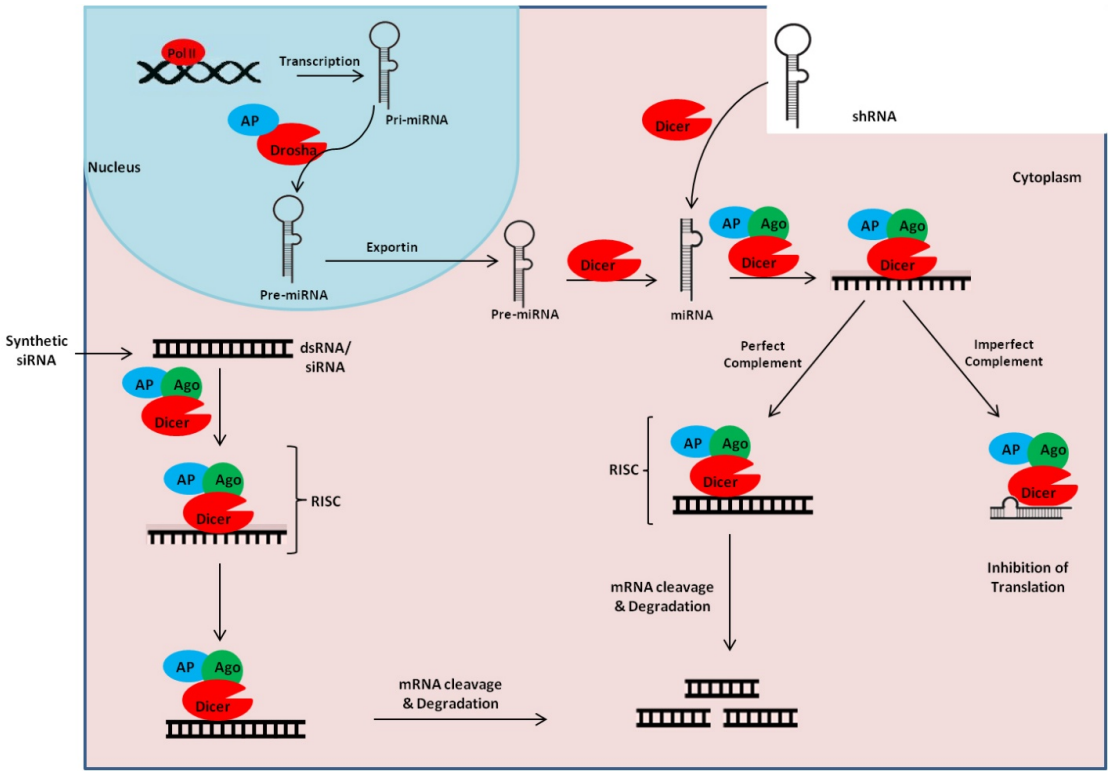


**Figure 4:** Model for RNA Silencing, an ordered biochemical pathway which is triggered by dsRNA of viral origin. The source of dsRNA is either the synthetic siRNA or pre-microRNA. Genome encoded pri-miRNAs are processed by Drosha

(an RnaseIII enzyme) into pre-miRNAs which are exported in the cytosol. dsRNA (siRNA or miRNA) subsequently joins Dicer, Ago and some other accessory proteins located in the cytosol forming RISC (RNA induced Silencing Complex). The degree of complementarity between the RNA silencing molecule and its cognate target determines the fate of the mRNA: blocked translation or mRNA cleavage/ degradation.

RNAi is an immune system in plants which is directed against viruses (Baulcombe, 2004; Wang et al., 2012). Upon viral attack, long dsRNAs are produced from the replication intermediates of viral RNAs which act as substrate for an endonuclease termed Dicer which is located in the cytosol (Tang *et al*., 2003). Dicer recognizes these dsRNA and cleaves them into duplex siRNA (21-25 nt) (Hamilton *et al*. 2002). siRNA duplex comprised of two strands; the strand complementary to target mRNA is guide strand and the other is passenger strand. The guide strand of short siRNA duplex is incorporated into the RNA-induced silencing complex (RISC) and then siRNA programmed RISC degrade viral RNA. As the RISC complex encounters a foreign mRNA which could of virus origin, it has two consequences. 1) If the homology of guide strand and target mRNA is 100% then perfect complement form between them, resulting in mRNA cleavage and subsequent degradation as shown in figure 4. This degradation is homology dependant and requires 100% complementarity between siRNA and the cognate viral mRNA or 2) in case of imperfect complement, where few mismatches exist between guide strand of RISC and target mRNA, translation of target mRNA is inhibited.

Same mechanism operates in microRNA triggered gene silencing. miRNAs processed from stem loop precursors (shRNA and/or hpRNA) and then requires Dicer activity (Tijsterman and Plasterk, 2004) followed by RISC assembly and subsequent degradation of homologous RNA in a sequence specific manner.

RISC is a combination of Dicer (an endonuclease enzyme), some accessory proteins namely argonaute (Ago1,4,6,9; catalytic endonucleases) and RNA binding proteins (PDR), and some trans-acting RNA-binding proteins (TRBP) (Gregory *et al*. 2005; Schwarz *et al*. 2003).

Stability of RNAi induced silencing is based on enzymatic methylation of siRNA. This reaction is catalyzed by the enzyme methyltransferase (HEN1) which methylates the siRNA at 3' end hereby preventing it from oligouridylation and subsequent degradation (Li *et al*. 2005).

When RNAi is induced at one site in plant, a mobile signal is generated which spread cell to cell and systemically throughout the plant (Fagard and Vaucheret, 2000) and make RNAi response obvious in distant tissues of the plant. This silencing signal moves inside plant either through the intercellular spaces called plasmodesmata or through the phloem (Voinnet and Baulcombe, 1997; Voinnet *et al*. 1998). It is proposed that in the cell to cell movement of silencing signal, short siRNAs are involved (Himber *et al*. 2003) while long siRNAs are involved in systemic movement (Hamilton *et al*. 2002). As RNAi mechanism is based on sequence specificity, it is evident that the signal could be an RNA molecule defined as aberrant RNA (aRNA), produced by the transgene itself (Palauqui *et al*. 1998). If these signals spread and the silencing condition is established ahead of a viral infection, viral RNAs are degraded before viral replication at the viral infection front (Voinnet *et al*. 2002).

### *4.7.2 Silencing Efficiency of Transgenes*

Initially to induce RNA silencing in plants, dsRNA transgene was co-expressed as sense and antisense transcripts of the target mRNA. The silencing efficiency achieved was far less as compared to the use of inverted repeat transgene as reported by Chuang and Meyerowitz (2000).

A small hairpin RNA (shRNA) is an RNA molecule that contains a fragment of a sense strand and an antisense strand, and a short loop sequence between the sense and antisense fragment thus

making a tight hairpin turn. After transcription, the inverted sequence is thought to make dsRNA that has the ability to suppress the expression of desired genes via RNA interference (Paddison *et al*. 2002; Frizzi and Huang, 2010). shRNA cassettes usually include a specific plant promoter and terminator sequences to control the expression of inversely repeated sequences of the dsRNA.

In general, gene silencing have proven fruitful with both sense- and antisense transgenes in plant cells (Bruening, 1998; Waterhouse *et al*., 1998), but it can be more efficiently achieved by utilizing shRNA cassettes (Hirai *et al*. 2007; Horiguchi, 2004; Watson *et al*., 2005). When shRNA cassette is delivered in the plant cells, dsRNA molecules are formed, which consist of a loop (single-stranded) and a stem region (double-stranded). Further, stem region is used by Dicer as a substrate and trigger RNAi mechanism (Hirai *et al*. 2007; Miki and Shimamoto, 2004). RNA Silencing mediated by the use of shRNA cassette enforces stable and heritable gene silencing (Paddison *et al*. 2002) as it utilizes the specific promoter to ensure that the shRNA is always expressed. Another reason which justify that the silencing efficiency can be more powerful when using shRNA cassette is due to the fact that dsRNA are being fed into a later step in the silencing pathway where they act as a substrate for Dicer (RNaseIII like enzyme) and therefore bypasses the step in which dsRNAs need plant encoded RdRps for their production (Prins *et al*., 2008).

RNA interference is conserved across kingdoms with same mechanism operating in all of them (Tabassum *et al*. 2011; Hamilton *et al*. 2002; Hamilton and Baulcombe, 1999; Plasterk, 2002). It proves to be one of an important tool for analysis of gene function in plants. Transgenic approach mediated by RNAi pre-programmed an existing antiviral defense in plants (Baulcombe, 1996). Plant viruses are the strong inducers of RNAi and a target as well. The simplicity and specificity of RNAi had made RNAi a routine tool for the generation of virus resistance crops.

### *4.7.3 RNAi Suppressors*

To counteract RNAi, many plant viruses encode some specific proteins called suppressors, which are produced in response to natural protection of plants against viruses. The discovery that plant viruses encode suppressors of gene silencing (Anandalakshmi *et al.* 1998; Beclin *et al.* 1998; Brigneti *et al.* 1998; Kasschau and Carrington, 1998; Vaucheret *et al.* 1998) provided a strong support that RNAi functions as a natural defense mechanism against viruses (Lindbo *et al.* 1993; Ratcliff *et al.* 1999). These virus-encoded suppressors act at various components of the RNAi pathway (specifically at DICER, Ago and RISC), allowing the virus to replicate and suppressing natural defense of plants (Li and Ding, 2006).

### *4.7.3 RNAi Suppressors: Molecular Basis of Mechanism*

In nature, various suppressors are encoded by different plant viruses, all of which are different from each other in their sequence and primary structure of protein. Virus encoded suppressor proteins are highly diverse in primary sequence and protein structure, although they may share some common mechanistic features. Among the many plant virus suppressors, helper component-proteinase (HC-Pro) is the best studied. It is encoded by potyviridae family and is reported to inhibit RNAi by acting on various steps involved in the maintenance of silencing at or upstream from the production of siRNA (Anandalakshmi *et al.* 1998; Brigneti *et al.* 1998; Llave *et al.* 2000; Mallory *et al.* 2001). In this review, we will emphasize on mechanism of action of suppressor HC-Pro, P19, 2b, P0 and Tobamovirus replicase protein.

### *4.7.4 Potyvirus HC-Pro:*

Helper Component Proteinase (HC-Pro) is a multifunctional viral protein involved in systemic infection of virus. HC-Pro participates in various biological processes as in viral replication and its intracellular systemic transport and also in the cleavage of the viral protein (Cronin *et al*. 1995; Kasschau and Carrington, 1995; Omarov and Bersimbai, 2010; Verchot *et al*. 1992) Above all, the

most significant biological role of HC-Pro is its contribution in RNAi suppression. It is reported that in infected plants, Helper Component Proteinase (HC-Pro) is the core factor in RNAi suppression (Anandalakshmi *et al*. 1998; Brigneti *et al*. 1998; Kasschau *et al*. 1998). Studies have revealed that central region of HC-Pro is necessary for suppressor activity while its N-terminal portion is not crucial for its activity.

Several mechanisms have been proposed through which HC-pro operates to suppress RNA mediated silencing in plants, including:

- Interaction of HC-pro with calmodulin protein (rgsCaM; regulator of gene silencing CaM) and inactivates the RNA-silencing pathway through an unknown mechanism at an intermediate step which involves both DICER and RISC (Anandalakshmi *et al*. 2000; Vionnet, 2005). As the expression of the viral proteins in infected plant cells is associated with accumulation of dsRNA, it strongly suggests that mechanism of action of HC-Pro is based on inhibition of Dicer enzyme (Dunoyer *et al*. 2004; Mallory *et al*. 2002).
- Another mechanism for HC-Pro action is based on its ability to form dimers and multimers that decrease the stability of siRNAs which are result of RNAi pathway (Plisson *et al*. 2003). Moreover, HC-Pro studies have revealed their functional impact on methylation of siRNA (Yu *et al*. 2006) and binding of dsRNA (Lakatos *et al*. 2006).

In addition to HC-Pro, there are several other RNAi suppressors which are reported in various virus families like suppressor protein P19 belongs to Tombus virus family; suppressor protein 2b is encoded by Cucumovirus family; Poleroviridae family encodes P0 suppressor protein; Tobamovirus replicase of tobacco mosaic virus; Closterovirus p21 protein is a strong suppressor of beet yellow virus; capsid protein of turnip crinkle virus has a suppressor p38; suppressor protein Tobra virus 16k belongs to tobra virus family and some virus families like Hordeivirus, tobra virus,carlavirus, furovirus, pecluvirus and barly strike mosaic virus encodes cystine rich proteins as suppressor proteins. Hordeivirus γb (cystine rich protein is specific for barley mosaic virus (BMV) (Omarov and Barsimbai, 2010).

Viral suppressors act on various stages of RNAi and have biochemical properties that enable viruses to effectively counteract the protective system of plants. Possible mechanisms through which suppressors operate include;

- Suppressor protein dimers like P19 form a complex with ds siRNA molecules thus making them unavailable for interaction with Dicer thus inhibiting RNAi. (Vargason *et al*. 2003; Ye *et al*. 2003). P19 also binds viral siRNAs hence making them inaccessible for RISC programming. On the other hand, P19 has the ability to suppress RNAi by preventing the activity of the HEN1 enzyme responsible for siRNA methylation. (Omarov *et al*. 2007; Pantaleo *et al*. 2007).
- 2b suppressor protein of Cucumovirus family blocks the RNAi cellular signals. Also it decreases the accumulation of 21-, 22-, and 24-nucleotide siRNA, by suppressing the activity of DCL enzymes. Moreover 2b interacts with AGO1 (the catalytic center of RISC) and this interaction leads to specific inhibition of enzymatic hydrolysis by the RNA nuclease complex (Diaz-Pendon *et al*. 2007; Zhang *et al*. 2006).
- A powerful suppressor P0 encoded by polerovirus acts at the level of the RISC while its expression leads to degradation of AGO1 protein by interacting with its PAZ domain (Bortolamiol *et al*. 2007).
- Tobamovirus replicase protein acts as RNAi suppressor and by binding to siRNA prevent its incorporation into the RISC (Csorba *et al*. 2007; Kurihara *et al*. 2007). In addition replicase is also reported to regulate the activity of HEN1 enzyme (Akbergenov *et al*. 2006; Vogler *et al*. 2007).

### 4.8 Advantages and Disadvantages of Antiviral Strategies

Antiviral strategies designed by plant pathologists and researchers have some advantages over the previous one but at the same time also render some potential disadvantages. In cross protection, resistance was met in some cultivars but the problem of virus recombination limits its further use. Similarly, in antisense RNA-mediated approach, resistance was relatively narrow ina sense that protection was specific only to the virus from which sequences were derived but not to strains with significant variation in corresponding transgene. In Pathogen derived resistance approach, scientists were met with mixed success. Resistance was strong often reaching to immunity and was specific to particular viral strain. However, in some cases, disease symptoms may be suppressed but virus replication is not affected, a situation that would do little to contain the further spread of the virus.

Subsequently, with the advent of homology-dependent gene silencing phenomena in plants, virus resistant plants generation entered into new era. RNAi technology offers several advantages over the previous antiviral strategies like it has the ability to target multiple gene family members with a single RNAi-inducing transgene, gene knockdowns due to RNAi are dominant, whereas insertional or other loss-of-function mutations are recessive. Similarly, RNAi is a powerful tool to induce loss-of-function phenotypes by inhibiting gene expression post-transcriptionally and activates the silencing process after the virus has entered the plant. Another advantage of RNAi is that it minimizes the risks associated with recombination between transgene RNA and viral RNA because short viral sequences of non coding regions are used. Conversely, RNAi has certain drawbacks including the presence of RNAi suppressors coded by viruses and has strong influence over the silencing process triggered by RNAi. Also, the presence of transgenes is required to maintain the silenced state, if they are lost or segregated by crossing, silencing is released (Stam *et al*. 1997; Mittelstein *et al*. 1994). Another disadvantage of RNA mediated resistance is that it is ineffective against viruses whose sequence differs from that of the transgene by more than 10% (De Haan *et al*. 1992).

### 4.9 Pitfalls and Advantages of Antiviral Transgenic Plants

Plant viruses as devastating pathogens cause substantial damage to crops by reducing their yield, vigor, and product quality. To overcome damages caused by viruses, the development of virus resistant crops seems to be a very effective approach. In strategies where virus derived genes are expressed in transgenic plants, there are potential environmental safety concerns regarding the constitutive expression of viral genes specifically concerned to the environment and to human health, heteroencapsidation, recombination, synergism, gene flow, impact on non-target organisms, and food safety in terms of allergenicity etc (Fuchs and Gonsalves, 2007). It is supposed that the infecting virus can interact with the expression product of inoculated milder virus strain in transgenic plants and thus can modify the biological properties of the existing virus, ultimately leading to creation of new virus species which may have novel pathogenic properties, host range and altered transmission specificity.

According to another perspective, possible outcomes of hetero-encapsidation and recombination are also reported in conventional plants which have mixed viral infection. And thus are not specific to transgenic plants resistant against viral infection. Therefore, it is not so much the occurrence but rather the consequences of hetero-encapsidation and recombination that should be of prime interest when assessing environmental risks of virus-resistant transgenic plants.

## 5. Conclusion

Several antiviral approaches discussed in this review have proved to confer effective resistance against a variety of plant viruses and it seems likely that outcome of some strategies in the form of virus resistant crops will benefit farmers as being cost effective by reducing input costs. Further, it

seems likely that the molecular biology and specifically RNAi has the potential to create and integrate new virus resistance factors in commercially important agricultural crops. The need now exists to understand the mechanisms behind these resistance strategies more precisely followed by utilization of this information to provide better resistance in transgenic plants. Furthermore, significant advances in elucidating the fundamental principles underlying resistance will lead to second and third generation genes that confer increased levels of sustainable resistance. The future challenge for scientists in this field is to develop strategies that broaden the breadth and increase the degree of resistance.

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