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Review

RNA Interference: A Natural Immune System of Plants to Counteract Biotic Stressors

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Abstract: During plant-pathogen interactions, plants have to defend the living transposable elements from pathogens. In response to such elements, plants activate a variety of defense mechanisms to counteract the aggressiveness of biotic stressors. RNA interference (RNAi) is a key biological process in plants to inhibit gene expression both transcriptionally and post-transcriptionally, using three different groups of proteins to resist the virulence of pathogens. However, pathogens trigger an anti-silencing mechanism through the expression of suppressors to block host RNAi. The disruption of the silencing mechanism is a virulence strategy of pathogens to promote infection in the invaded hosts. In this review, we summarize the RNA silencing pathway, anti-silencing suppressors, and counter-defenses of plants to viral, fungal, and bacterial pathogens.

Keywords: RNA interference; AGOs; DCLs; RDRs; pathogens; suppressors; resistance

1. Introduction

As sessile organisms, plants have to endure a range of adverse conditions imposed by various biotic and abiotic stressors that diversely affect growth, development, and yield of plants. The occurrences and severity of the stressors may vary depending on the locality and natural environment. In particular, biotic stresses caused by the living organisms largely depend on the availability of suitable environments that activate specific organisms to infect their hosts. Among different kinds of biotic stressors that have existed since ancient time, bacteria, fungi, nematodes, and viruses are capable of causing different types of plant diseases. In response to an attacker, plants activate a specific set of defense mechanisms or related pathways, leading to either increased resistance (limits pathogen multiplication) or enhanced tolerance (reduces the effect of infection) depending on the nature of pathogen and host. RNA interference (RNAi) or RNA silencing is one of the important defense mechanisms that protects plants from pathogen infection and it controls sequence specific regulation of gene expression.

The phenomenon of RNAi was first reported in the early 1990s and it was hypothesized that introduced genes potentially co-suppressed the endogenous genes [1], but soon it was observed that homologous RNA sequences caused the suppression of internal genes [2]. The term RNA silencing was first used in a study on an animal (*Caenorhabditis elegans*) and it was observed that introduction of sense or antisense RNA caused degradation of the *par-1* gene [3]. Later, it was reported that interference of double-stranded RNAs (dsRNA) was more significant in both the injected animals and their progenies [4]. After two years, it was found that dsRNAs could convert to shorter small interfering RNAs (siRNAs) and bind to their homologous target messenger RNAs (mRNAs) [5,6]. These siRNAs also guide and instruct the multicomponent RNA-induced silencing complexes (RISCs) to cleave the specific RNAs [5]. The RNase III family enzymes were found to be involved in the cleavage of dsRNAs and could produce putative guide RNAs [7].

The proposed RNA silencing mechanism starts with the production of 20 to 26 nucleotide (nt) small RNA (sRNAs) through a series of key components, such as Dicer-like protein (DCL), Argonaute (AGO) protein, and RNA-dependent RNA polymerase (RDRs) [8–10]. The DCL proteins generate sRNAs from a dsRNA precursor and then incorporates into RISCs [11]. On the basis of their origin and formation, these sRNAs are divided into siRNAs or microRNAs (miRNAs). AGO proteins perform the large part of RISCs, bind the sRNAs and interact with homologous RNAs, that affect DNA methylation, endonuclease activity, or translational repression of mRNAs [12]. RDR enzymes are responsible for the synthesis of dsRNAs using single-stranded RNAs (ssRNAs) as the templates, which are then further processed by Dicer-like (DCLs) proteins and start a new round of RNA silencing [13].

In line with the diverse roles of RNAi, researchers found a number of *DCLs*, *AGOs* and *RDRs* genes in different plant species, and described their roles in plant defense mechanisms. Due to the presence of a vast number of pathogens and their threats to plants, researchers continuously study plant RNAi mechanisms as a defensive mechanism against the pathogens. The number of pathogens and RNAi pathway complexity make it difficult to understand the complete mechanism. In this review, we summarized the plant RNAi responses to various pathogens in the light of already published work and described it in a schematic way to simplify and understand the whole mechanism. Here we first, discuss the structure and nomenclature of the main three proteins of the RNAi pathway. Second, the role of these proteins and their cofactors in silencing of pathogens derived siRNAs and how they counteract the anti-silencing suppressors during plant–pathogen interaction are discussed. Finally, we summarize the recent progresses of RNAi machinery in plant immune response to viral, fungal, and bacterial diseases.

2. RNAi Pathway Components

2.1. Dicer-Like Proteins (DCLs)

DCLs are the RNase III family of endoribonucleases that contain DExD-box Helicase-C, Piwi-Argonaute-Zwille (PAZ) domain, the Domain of unknown function 283 (DUF283), RNase III, and dsRNA-binding domains (dsRBDs) [14,15]. These proteins perform the initiation stage of the RNAi mechanism, in which dsRNAs are cleaved into small RNAs 21–24 nt in length [14]. The helicases are enzymes that induce the separation of double-stranded nucleic acids, utilizing the free energy and hydrolyzing a nucleotide triphosphate to displace bound proteins [16]. A functional DExD/H-box helicase domain is also required for the efficient production of DCLs-dependent sRNAs production [17]. PAZ domain has a phosphate-binding pocket composed of arginine components that recognize the 5′ monophosphate of pre-miRNAs and are required for cleavage of dsRNAs [18–20]. DUF283 acts as an annealer that assists hybridization between the complementary strands of nucleic acids [21]. The function of RNase III domain is to cleave dsRNA, leaving the 2 nt overhang at 3′ end of the product [22,23]. The N- or C-termini of dsRBDs participate in the regulation of the protein nucleo-cytoplasmic distribution and also have the capacity to bind to dsRNAs [24,25]. The specific function of each domain varies depending on the Dicer protein.

Dicer proteins are conserved across the plant kingdom and up to date, various DCL proteins have been identified in different plant species, which are classified into four distinct clades. Plant DCLs produce different size of sRNAs: DCL1 produces 21 and 22 nt sRNAs from the short imperfectly hairpins RNAs, while DCL2, DCL3, and DCl4 produce 22 nt, 24 nt and 21 nt, respectively from long perfectly paired RNAs [26]. DCL1 is involved in the generation and processing of miRNAs [27,28]. Viral dsRNAs processed by DCL2 and DCL3 are required for chromatin modification and also produce heterochromatic siRNAs (hc-siRNAs) [29,30]. DCl3 is also an important element of RNA-directed DNA methylation (RdDM) pathways and it processes RDR2 generated dsRNAs into siRNAs [31]. DCL4 processes *trans*-acting small interfering RNAs (ta-siRNAs) and can be a substitute for DCL1 and DCL2 when these two are missing [32–34]. The product of DCLs or initiation stage is loaded into the RISCs for further processing.

2.2. Argonaute (AGO) Proteins

AGO proteins are highly specialized sRNA-binding modules and are considered to be the essential components of RISCs in silencing pathways [11,35]. AGO proteins perform the effector phase of silencing and the small RNAs produced in the initiation stage are loaded into AGO proteins to guide sequence-specific regulation of gene expression. Structural analysis shows that AGO proteins contain three conserved domains, such as PAZ, Middle (MID) and P-element induced wimpy (PIWI) domains. The N-terminal domain is composed of the N-terminal region and PAZ domain which facilitate the separation of small RNAs and anchor the 3′ end of the bound small RNA, respectively. The C-terminal lobe contains MID and PIWI domains, and a binding pocket at the junction of these domains that anchors the 5′ end of small RNAs [36], however, the PIWI domain can function similarly to RNase H that cleaves target mRNA [37].

Plant AGO proteins are classified into four groups and each group performs a specific function. These proteins are involved in DNA methylation and epigenetic silencing [38,39], miRNA-mediated silencing pathways [40], and stage transition of plants from the juvenile to the adult stage of growth [41]. The most important function of AGO proteins is to enhance the defense and immunity of plants through cleavage of the loaded small RNAs. Notably, AGO proteins require heat shock proteins (Hsp70–Hsp90) to chaperone and hydrolysis ATP for the loading process and conformational changes [42,43]. The size and type of the 5′ nucleotide help in the sorting of sRNAs to specific AGO proteins [35]. The less stable 5′-end pairing ds-sRNA molecule is retained within the AGO while the others are eliminated [44,45]. The action of specialized AGO proteins and small RNAs divide the plants RNAi pathways into transcriptional gene silencing (TGS) and posttranscriptional gene silencing (PTGS). AGO4, 6, and 9 have a role in TGS, while AGO1, 2, 3, 5, 7, and 10 are involved in PTGS [35]. AGO4 clade also participates in the RdDM pathway and binds the 24 nt dsRNAs produced by RDR2 and DCL3 [46].

2.3. RNA-Dependent RNA Polymerase (RDR) Proteins

The third phase of RNAi pathways is an amplification of silencing, performed by RDRs, which convert ssRNAs to dsRNAs and these dsRNAs are again processed by DCLs, leading to a new cycle of RNA silencing. In sRNAs, the phased and repeat-associated siRNAs depend on RDRs for biogenesis, while miRNAs and hairpin-derived sRNAs are RDRs independent RNAs. The activity of RDR was first studied in Chinese cabbage during 1971 and cDNA was isolated from tomato [47,48]. RDRs are considered as the first identified component of plants small RNAs biogenesis pathways and characterized by a unique RNA-dependent RNA polymerase catalytic domain (RdRp) [49,50]. RdRps belong to the Structural Classification of Proteins (SCOP) and initially, these proteins were identified as an enzyme in the RNA viruses that participated in the replication of the virus genome [51]. RdRps also have a role in the maintenance of genome integrity, RNA-template formation, PTGS, and defense against external RNA or DNA [52,53]. RDR proteins of eukaryotic organisms are divided into three main classes, RDRα, RDRβ, and RDRγ. RDRα exists in all three kingdoms. Animals comprise the RDRβ protein missing the RDRγ, whereas RDRγ is present in plants, and fungi contain both RDRβ and RDRγ proteins [52]. The phylogenetic analysis divides the plants RDRs into following subclasses, RDR1, 2, 3, and 6 [54,55]. In *Arabidopsis*, RDR proteins comprised six RDRs members, among them RDR-1, -2, -6 and RDRs -3, -4, -5 belong to RDRα and RDRγ clades, respectively.

RDR1, RDR2, and RDR6 are extensively studied in the plant kingdom. The general function of RDRs is further categorized to an individual member of the groups, the RDR1 is involved in the amplification of exogenous nucleic acid and defense against insects and pathogens [56–58]. RDR2 is an important component of the RdDM pathway and is also required for biogenesis of siRNAs and nuclear RNAi [29,59]. Interaction of RDR2 with Jumonji (JmjC) domain-containing protein (JMJ24) promotes RNA-based chromatin silencing in higher plants [60]. However, it was also reported that during sense transgenes post-transcriptional gene silencing (S-PTGS), RDR6 triggered DNA methylation of the transcribed region and RDR2 is not required for this type of methylation [61,62]. The function of RDR6–RdDM has been correctively reestablished as it triggers transposable elements (TEs) methylation

and epigenetic silencing [63]. RDR6 is also involved in initial signal perception and control of miR165/166 accumulation during normal plant development [64,65]. RDR6 processes aberrant non-spliced pre-mRNAs and channels it to the RNA silencing pathway [66]. On the basis of RDRs dependent biogenesis, the sRNAs fall into two major categories, phased and repeat-associated short interfering RNAs are known RDR1/2/6-dependent sRNAs, while miRNA and other hairpin-derived sRNAs are RDRs independent sRNAs. Recently, 38 RDR1/2/6-independent sRNA-producing loci were identified in *Arabidopsis* that do not fit into any currently understood schema for plant sRNA biogenesis [67]. The most important role of plant RDRs proteins is to collaborate with other components of RNAi machinery and provide defense against invading nucleic acids. Different DCLs, AGOs, and RDRs proteins identified in plant species are listed in Table 1.

Table 1. Number of Dicer-Like protiens (DCLs), Argonaute (AGOs) and RNA-dependent RNA polymerase (RDRs) identified in various plant species.

Plant Species	DCLs	AGOs	RDRs	References
Arabidopsis thaliana	4	10	6	[11]
Brassica napus	13	28	16	[68,69]
Capsicum annuum	4	12	6	[70]
Coffea canephora	9	11	8	[71]
Cucumis sativus	5	7	8	[72]
Glycine max	7	21	7	[73]
Nicotiana benthamiana	4	9	3	[74]
Oryza sativa	8	19	5	[75]
Phaseolus vulgaris	6	17		[76]
Salvia miltiorrhiza	5	10	5	[77–79]
Setaria italica	8	19	11	[80]
Solanum lycopersicum	7	25	6	[55]
Solanum tuberosum	7	11	7	[81,82]
Sorghum bicolor	5	14	7	[73]
Vitis vinifera	4	13	5	[83]
Zea mays	5	18	5	[54]

3. Plant RNA Silencing and Viruses

3.1. Plant RNA Silencing Machinery against Viruses

Viruses need a vector for transmission from one host to another, and they use host resources for their reproduction and dissemination. In plants, viruses not only produce local lesion but also a systemic infection that causes malformation, chlorosis, and stunted growth. The majority of plant viruses possess single or double stranded RNA genome except for a few families of DNA viruses. Unlike other pathogens, viruses multiply within the host cells and, hence, the RNAi pathways play a crucial role in anti-viral defense. In plants, the primary targets of the RNAi machinery are viruses with RNA genomes, which produce dsRNA intermediates during the reproduction process. DCL2 and DCL4 directly process the virus RNAs and produce 22 and 21 nt siRNAs respectively, which is further loaded to the AGO1 and AGO2 complex for cleavage [84]. In the case of DNA viruses, dsRNA phase is absent in their replication cycle, and RDR6 and Suppressor of gene silencing 3 (SGS3) are required for RNA silencing [85]. DNA viruses also require all four DCLs proteins to produce 21, 22, and 24 nt siRNAs in the host cells [86].

Virus multiplication increases accumulation of virus-derived siRNAs (vsiRNAs) during infection, and many studies have shown that virus inoculation induces the transcription level of various RNAi genes in different plant species. For example, Cucumber mosaic virus (CMV) enhances RDR1, RDR2, and RDR3 in *Salvia miltiorrhiza* [78], Tomato yellow leaf curl virus (TYLCV) up-regulates most of the tomato DCLs, AGOs, and RDRs [55], CMV, Potato virus Y (PVY) and Tobacco mosaic virus (TMV) infections up-regulate *CaDCL2*, *CaDCL4*, *CaAGO2*, and *CaAGO10b* in *Capsicum annuum* [70]. Similarly,

CMV infection in *Arabidopsis* results in the accumulation of CMV-vsiRNAs of 21-, 22-, and 24 nt, wild-type and *dcl1*, *2*, and *3* single mutants produce 21 nt species after infection, which is abolished in the *dcl4* mutant [87]. Whereas, *dcl2-1* and *dcl3-1* mutants lack 22 nt and 24 nt vsiRNAs after CMV infection [88], revealing that CMV infection requires DCL2, DCL3, and DCL4 for the accumulation of various classes of vsiRNAs that are needed for antiviral silencing. DCL2 and DCL4 are crucial against RNA viruses, and viral RNA accumulation and systemic infection are more effective in both DCL2 and DCL4 inactive plants [89–91]. DCL2 is essential to promote the cell-to-cell spread of virus-induced RNA silencing, while DCL4 participates in cell-autonomous intracellular silencing and inhibits intercellular silencing, the DCL2 and its 22 nt vsiRNA may also substitute for the DCL4 when it is absent or suppressed by viruses [92,93]. The 24 nt siRNA produced by DCL3 is not enough against invading RNA, and, thus, the production of 21- and 22 nt species is needed for an effective silencing process. However, accumulation of the 24 nt transgene-derived siRNAs (t-siRNAs) are associated with plant immunity against crinivirus [94], which shows that DCL3 play minor role in antiviral defense against RNA viruses.

In case of DNA viruses, the DCL3 and its cognate 24 nt vsiRNAs are associated with RdDM to protect the plant from infection [95,96]. In addition to RdDM, hyponastic leaves 1 (HYL1) family proteins and Hua enhancer 1 (HEN1) are involved in DCLs induced biogenesis of DNA virus vsiRNAs [86]. HEN1 also methylates all endogenous sRNAs and protects them from uridylation activity [97]. It was reported that *Hen1* mutants were more susceptible to CMV and TCV virus infections [92,98]. In the Cabbage leaf curl virus (CaLCuV), DCLs produce different classes of virus siRNAs, but the dsRNA precursors for these vsiRNAs are produced by RDRs independent pathways and do not require RNA polymerases IV and V (Pol IV and Pol V) [99]. These are generated by RNA polymerase II through transcription of viral DNA [96]. Like RNA viruses, DCLs also function in the antiviral mechanism of DNA viruses. TYLCV inoculation induces the expression of *SlDCLs* and silencing of the *SlDCL2*/*SlDCL4* increases the sensitivity of tomato to TYLCV infection [100], confirming that 21 and 22 nt siRNA production is also required for the plant–DNA viruses silencing mechanism.

Plants also contain special double-stranded RNA binding (DRB) proteins that promote DCLs for the precise production of small RNAs. DRB proteins are non-catalytic factors having double-stranded RNA binding motif (dsRBM). In *Arabidopsis*, the *AtDRB2*, *AtDRB3*, and *AtDRB5* are involved in the miRNA biogenesis pathway [101,102], and *AtDRB3* and *AtDCL3* together affect the methylation of the viral genome [103]. Interactions of DCL1 and DRB1 are required for the efficient production and loading of miRNA, while DRB2 binds DCL1 for miRNA biogenesis and improves silencing action [104,105]. DRB2 is also involved in the recruitment of repressing epigenetic factors that fine-tune the transcription at targeted loci, and the loss of function of DRB2 accumulates a higher amount of RNA polymerase IV-dependent siRNAs, suggesting that the transposable element transcript would be converted by the RdDM pathway [106,107]. Similarly, DRB4 facilitates the DCL4 activity for biogenesis of miRNAs and antiviral silencing in plants. DRB4 and DCL4 collectively participate in Turnip yellow mosaic virus (TYMV)-derived siRNA production and antiviral responses [108], but in the absence of DRB4, other members of this family might assist DCL4 in the biogenesis of 21 nt vsiRNA [109]. However, the interactions of some DRB members (DRB7.1) with DRB4 or DRB complex antagonize the production of siRNAs and RNase III activity in plants [110]. In contrast, the DRB7.2 directly interacts with DRB4 and participates in the epigenetically activated siRNAs pathway [111]. Recently, it was reported that the interaction of conserved Forkhead-associated (FHA) domain-containing protein DAWDLE (DDL) with DCLs is required for the biogenesis of siRNAs. The DDL particularly interacts with DCL3 and improves its activity [112]. Moreover, DDL has also been shown to be required for development and immunity [113,114].

The siRNAs produced by DCLs are incorporated into AGOs, which is an important downstream step for silencing of external nucleic acid. In plants, various AGOs were identified, and functions of few members against multiple viruses were revealed. Genetic analysis of the *Arabidopsis* AGO

proteins showed that only AGO1, 2, and 7 are involved in the virus resistance and have a role in RNA silencing and restriction of the virus [115,116]. Among them, AGO1 is considered as the first activation layer and AGO2 as a second defense layer to restrict virus accumulation after AGO1 is suppressed by the virus suppressor. The activation of the second defense also indicates that the first layer is lost due to the suppression of AGO2 by AGO1 through miR403 [117]. Similarly, AGO5 performs an antiviral role in the absence of AGO2. However, both have the ability to bind vsiRNAs. The *ago2 ago5* double mutants are more susceptible to the virus than single mutant [118,119]. AGO1 has the capability to target compact structure viral RNAs, while AGO7 favors less structured RNAs and both are involved in the silencing of Turnip crinkle virus (TCV) RNAs [120]. *Ago1*, *ago2*, and *ago7* (knockdown) mutants show increased hyper-susceptibility to CMV, TCV, TMV, potato virus X (PVX) and ringspot virus [117,120–124]. Similarly, plants require AGO proteins (AGO4, AGO6, AGO9) for systemic silencing where AGO4 plays a crucial role in maintaining or activating the expression of stress-responsive genes via chromatin modification or preventing cryptic transcription [125,126]. This clade is localized in the nucleus and the 24 nt siRNAs associated to AGO4 are dependent on DNA-dependent RNA polymerase IV, RDR2, and DCL3, showing the binding of AGO4 to long coding RNAs [39,127,128] (Figure 1). In case of *Plantago asiatica* mosaic virus (PlAMV), the virus changes the AGO4 localization from nuclear to cytoplasmic distribution and AGO4 target virus RNAs are independent of RdDM components (DCL3, Pol IV, and Pol V) [129]. This independent class of siRNAs mainly originates from transgenes, transposons, and intergenic sequences [130].

Previous studies reported that *ago4* mutants are more susceptible to Tobacco rattle virus (TRV) and Beet curly top virus (BCTV) [131–133]. AGO4 is also involved in host transcriptional response and resistance against CMV and PVX viruses [134,135]. Recently, researchers have identified many new members of the AGO proteins in different plant species, but their functions still remain unknown. Therefore, research in the current direction may be helpful to better understand the effector phase of silencing.

The host RDRs proteins use the viral primary siRNA molecules as a primer and convert them into long dsRNAs, leading to the amplification of siRNA signals. RDR1, RDR2, and RDR6 are considered important members that act in the various biological processes of RNA silencing. Recently, it was found that Tobacco mosaic virus-coat protein (TMV-CP) accumulation was less in the *CaRDR1* overexpressing tobacco lines following TMV infection. Furthermore, TMV inoculation enhanced transcript levels of *AGOs* and *DCls* genes in the *RDR1* expressing lines [136]. Similarly, *MdRDR1* transgenic tobacco plants enhanced resistance against TMV, Sunn hemp mosaic virus and Turnip vein-clearing virus [137,138]. In the case of CMV, the siRNAs production depends on the RDR1, and these secondary vsiRNAs are important for antiviral silencing [88]. Silencing of *RDR1* or knockout *rdr1* mutants result in a higher level of virus RNA and reduces resistance in pepper, *Arabidopsis*, maize, and tomato plants [136,139–141], however, the resistance of potato to PVX and PVY is not compromised [142]. As we mentioned, RDR2 protein along with DCL3 is involved in the production of 24 nt siRNAs, and loss of RDR2 function causes extensive changes in the expression of genes, transposons and 24 nt sRNAs [61]. This protein is considered as the main component of the RdDM pathway and required for cell-to-cell silencing and the reception of the long-distance mRNA silencing signal [49,64]. RDR2 is also involved in TGS pathway and in antiviral defense [50,143,144]. Loss of RDR2 function results in the up-regulation of certain elements that are members of *Ty3*/Gypsy-like super-family of retrotransposons and these elements are transcriptionally silenced via the RdDM pathway. *Ty-1* and *Ty-3* are resistance allele of wild-type tomato against the TYLCV virus, these alleles encode the DFDGD motif and belong to the RDRγ clade [145,146]. It is believed that various *Ty* genes are involved in RNA silencing and viral genomes cytosine methylation [145,147,148]. *Ty-3* and *Ty-4* significantly lower the Tomato mottle virus (ToMoV) disease severity, while *Ty-6* and *Ty-5* provide resistance against TYLCV [149]. Genetic analysis of interspecific crossing between *Solanum habrochaites* and cultivated tomato show that a nucleotide-binding domain and leucine-rich repeat (NB-LRR) containing gene, *TYNBS1*, is synonymous with the *Ty-2* gene and strictly co-segregated with TYLCV

resistance [150]. Similarly, the *Ty-1* and *Ty-3* markers were detected in the TYLCV resistant tomato lines, but not in the susceptible lines [151].

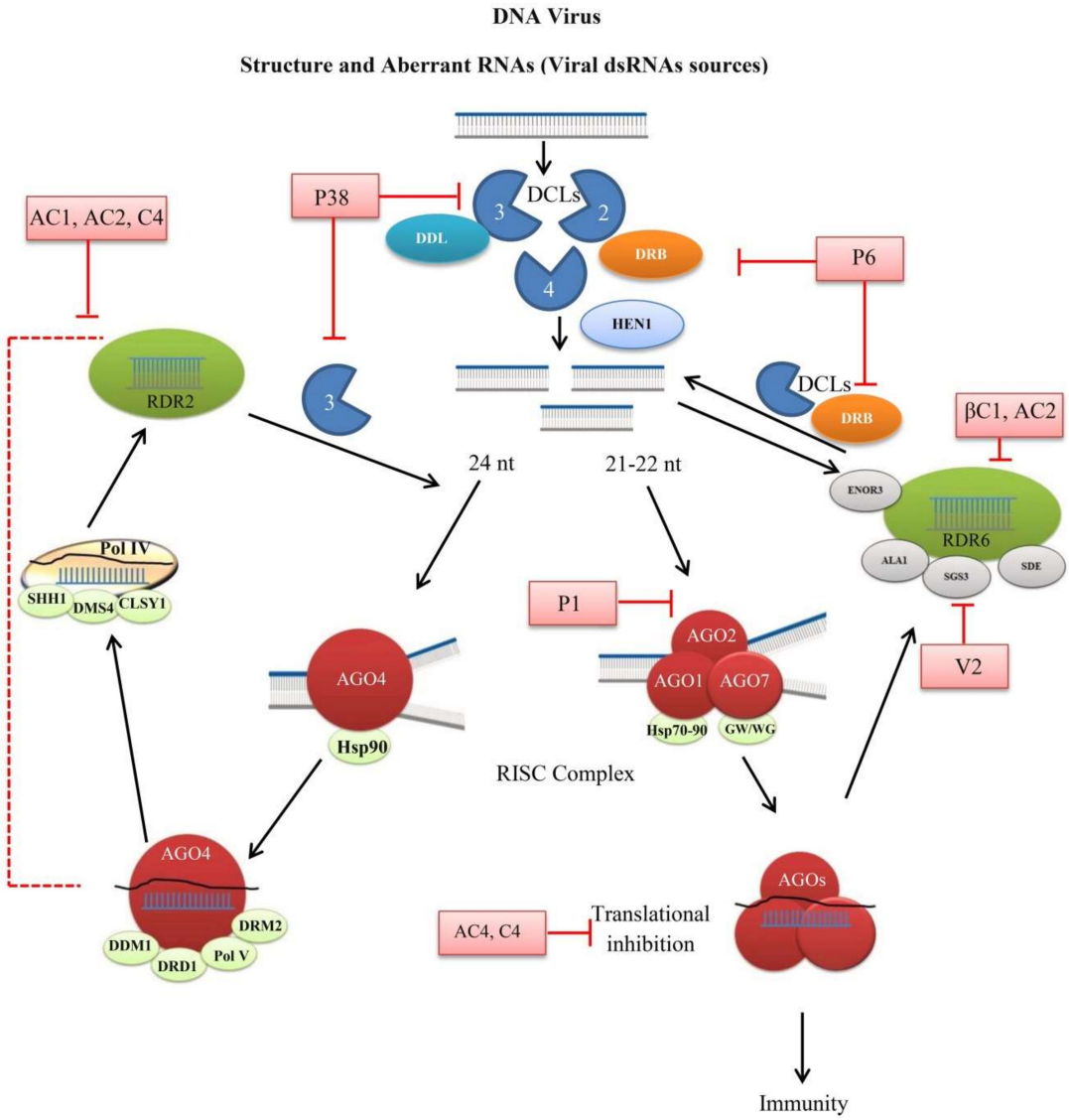

Figure 1. Schematic diagram of plant RNA silencing against DNA viruses. Plant Dicer-like proteins (DCLs) generate 21, 22 and 24 nt siRNAs from viral dsRNA. The 21 and 22 nt siRNAs are incorporated into Argonaute (AGOs) containing RNA-induced silencing complex (RISC) for silencing or translational inhibition. The RISC product may enter into the amplification line and produce secondary small interfering RNAs (siRNAs) through the actions of RNA-dependent RNA polymerases (RDRs) and cofactors. While, the 24 nt siRNAs are methylated through RNA-directed DNA methylation (RdDM) pathway including AGO4, RNA polymerase (Pol IV and V), RDR2 and finally processed by DCl3. Viral silencing suppressors (VSRs) block the RNAi mechanism by inhibiting the function of various components (pink box). Double-stranded RNA binding (DRB) proteins, Hua enhancer 1 (HEN1), DAWDLE (DDL), Heat shock proteins (Hsp), Silencing defectives (SDE), Suppressor of gene silencing 3 (SGS3), Aminophospholipid ATPase 1 (ALA1) and Enhancer of RDR-3 (ENOR3) proteins. Glycine/Tryptophane (GW), DNA-dependent RNA polymerases (Pol IV and V), Domain rearranged methyltransferase 2 (DRM2), Defective in RNA-directed DNA methylation 1 (DRD1), Deficient in DNA methylation 1 (DDM1), Classy 1 (CLSY1), Defective in meristem silencing 3 (DMS3), and Sawadee homeodomain homolog 1 (SHH1).

The RDR2 also competes with RDR6 for RNA substrate and antagonizes RDR6, because RDR2 is functionally more active in the presence of counteractive siRNA; as a result, *rdr2* mutants show increased efficiency of sense transgenes post-transcriptional gene silencing (S-PTGS) [62]. As systemic and intracellular PTGS requires RDR6 that restricts pathogen at both the systemic and single cell level [152]. *NbRDR1* expression up-regulates in *RDR6*-silenced plants infected with CMV-2b non-expression mutant, and RDR1 synergistically functions with RDR6 to facilitate immune responses [153]. RDR6 activity is assisted by protein cofactors Suppressor of gene silencing 3 (SGS3) and Silencing defectives (SDE3 and SDE5) [50,154–156]. Due to RNA silencing, the apical meristem of plants shows resistance to viral infection, but in case of *Arabidopsis rdr6* mutant, the CMV wild and mutant type can cause severe symptoms with minimum antiviral activities in the apical meristem [157]. Recently it was found that membrane-localized flippase Aminophospholipid ATPase 1 (ALA1) and Enhancer of RDR-3 (ENOR3) proteins cooperate with RDR6 to promote antiviral immunity. *Arabidopsis* double mutants *ala1-2 rdr6* and *enor3-1 rdr6* exhibit severe symptoms after inoculation with CMV. Both ALA1 and ENOR3 inhibit the accumulation of CMV and functions additively with RDR1 and RDR6 to mediate plant immunity [158,159]. Tobacco RDR6 expressing lines show enhanced resistance to PVY while on the other hand, its silencing results in the accumulation of viral RNAs and increased susceptibility to the Chinese wheat mosaic virus (CWMV), Rice dwarf phytoreo virus (RDPV) and Rice stripe virus (RSV) [160–163]. In some viral infections, the accumulated viral siRNAs are RDRs independent and act as a poor template for production of secondary siRNAs. For instance, the accumulated vsiRNAs of Pelargonium line pattern virus (PLPV) and CaLCuV are not associated with the RDRs activity [96,164]. Taken together, RNAi mechanism is an important plant defense mechanism that mimics viral pathogens, but the research body is relatively less regarding the other members of these gene families and, therefore, more study is needed to unravel the functions. The reported function of various components of plants RNAi pathway against the RNA viruses and suppression by silencing suppressors is presented in Figure 2.

3.2. Viral Suppressors Block Plant RNA Silencing

To neutralize the effect of RNAi, well-adapted plant viruses have developed viral suppressors of RNA silencing (VSRs), which largely attenuate plant defense against such type of viruses [165]. It is suggested that the VSRs are essential for successful viral infection and most of the viruses contain at least one VSR that blocks the RNA silencing at any step. In CMV infection, the accumulation of various viral siRNAs produced by DCL1, DCL2, and DCL3 is reduced by the expression of 2b suppressor [88]. Similarly, the P6 of Cauliflower mosaic virus (CaMV) interacts with DRB4, an important partner of DCL4 and affects the viral siRNA processing [166]. In Turnip crinkle virus infection, P38 suppresses DCLs, but its main target is DCL4, as DCL4 produces silencing signal to restrict virus exit from vascular bundles and P38 inhibits this signaling [167]. In plants, Glycine/Tryptophane (GW)-containing proteins are required for miRNA and siRNA RISC function. TCV P38 and Sweet potato mild mottle virus (SPMMV) encode GW P1 proteins that interact with cellular AGOs and isolate AGO1 from associated siRNA and miRNA effector complexes preventing loading and RNA silencing [168,169]. CMV 2b and TRV 16K suppressors restrict the initial formation of miRNA- and siRNA-guided RISCs and bind to AGO4 to prevent target RNA cleavage, inhibit AGO4 slicer activity, and the methylation of numerous loci [133,135]. Moreover, 2b and P0 proteins inhibit the RISC activity through interaction with the PAZ domain of AGOs and directing its degradation [170–172]. The widespread distribution of P0 in host cells hijacks the F-box containing complex or SKP1-CUL1-F-box (SCF) machinery to stop silencing by destabilizing AGO and results in severe disease infections [172,173]. In SPMMV, P1 suppressors block target RNA binding to AGO1 and the zinc finger domain of P1 performs the suppression activity [174]. The Pelargonium flower break virus coat protein (PFBV-CP) and Tomato bushy stunt virus (TBSV) P19 suppressor also prevent the incorporation of the viral siRNAs into the RISC complex [175–177], however, P19 specifically impairs loading into AGO1 but not AGO2 [178], and TBSV *p19* mutant is highly susceptible to RNA silencing [179,180]. Citrus tristeza virus (CTV) P20 and P23 suppress

salicylic acid (SA) and the RNA silencing defense pathways of orange plants [181,182]. The existence of more suppressor proteins in one virus seems to be lead in targeting various host defense pathways at the cellular level for more successful infection. Olive mild mosaic virus coat protein (OMMV-CP) and P6 show suppressor activity with complementary manner and silencing of both suppressors result in significant reduction of viral accumulation and symptoms compared to single silencing [183].

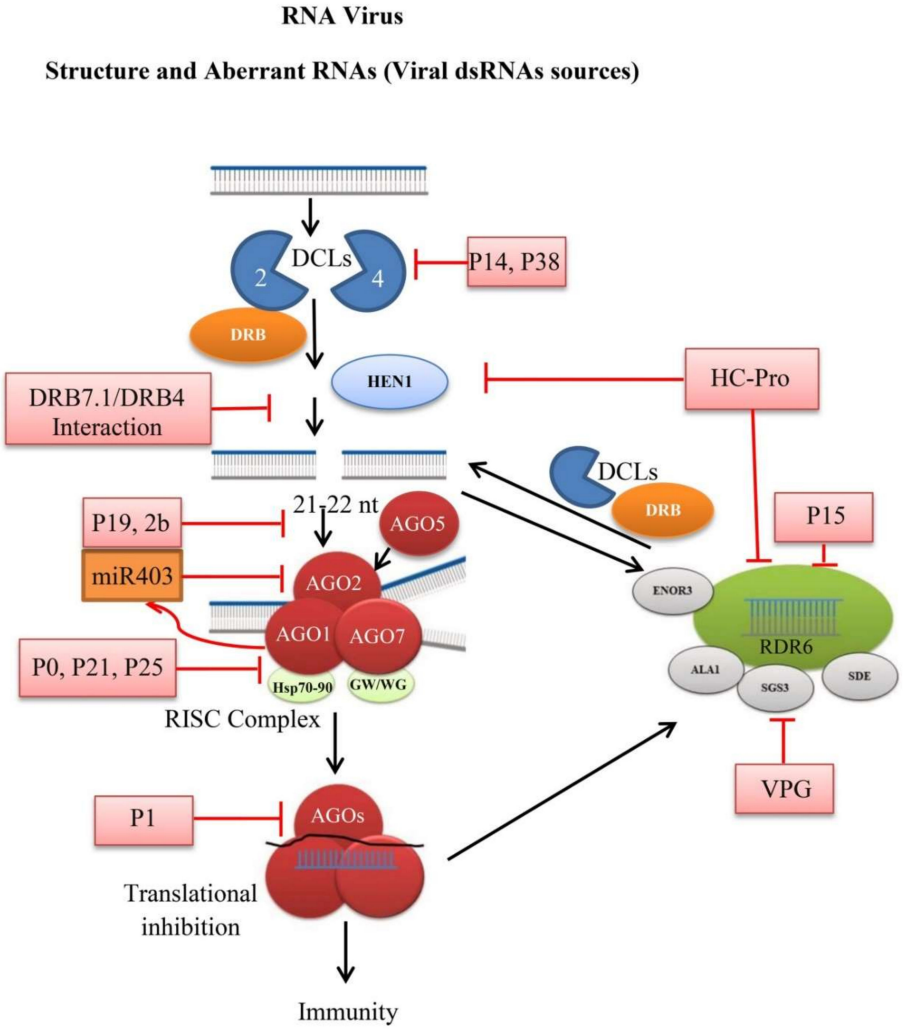

Figure 2. Schematic diagram of plant RNA silencing against RNA viruses. DCLs generate 21–22 nt siRNAs from viral dsRNA. The siRNAs are incorporated into AGOs containing RISC for silencing or translational inhibition. The RISC product may enter the amplification line and produce secondary siRNAs through the actions of RNA-dependent RDRs and cofactors. VSRs can block the RNAi mechanism by inhibiting the function of various components (pink box). AGO2 suppression by AGO1 through miRNA (orange box). DRB proteins, HEN1, Hsp, SDE, SGS3, ALA1, and ENOR3 proteins.

Virus movement and replication in the host cell need to suppress RDRs activities to stop the silencing amplification and distinct signaling. In tobacco plants, symptom development after inoculation of two different begomoviruses depends on the interaction of virus AV2-encoded pre-coat protein and host RDR1. The pathogen AV2 represses the RDR1-mediated silencing for the establishment of disease symptoms in the hosts [184]. Helper component proteinase (Hc-Pro), P19, and P38 altered accumulation and reduce primary siRNAs level to suppress the RDR6-directed transitive RNA silencing. Similarly, P19, P38, and P15 constrain the RDR6-dependent *cis*- and *trans*-acting inhibitory effects that normally restrict the accumulation of PVX in *Arabidopsis* [185]. The V2 protein of TYLCV suppresses the RNA silencing pathway by either directly interacting with RDR6 cofactor

(SGS3) or enhancing the accumulation of Calmodulin-like proteins (CaM) that cause SGS3 degradation through autophagic pathways [186,187]. Notably, a mutation in amino acid on position 71 affects the self-interaction, aggregation, and pathogenicity of V2 [188]. Tomato spotted wilt virus NSs proteins can replace HC-Pro–deficient potyvirus function and favor the development of local and systemic symptoms [177]. Likewise, mungbean yellow mosaic Indian virus (MYMIV) AC2 protein suppresses RNA silencing mechanism by interacting with the host RDR6 protein [189].

In addition to the suppression of host RNAi machinery, certain suppressors are involved in virus replication and movement, and they need a host factor for successful infections. Cysteine-rich protein (CRP), 6K1 and 29K are important elements that contribute to RNA silencing suppression in the context of virus replication at early stage of infection, whereas Triple gene block protein 1 (TGBp1), HC-Pro, P3, and P3N-PIPO are required for viral cell to cell and long-distance movement [190–194]. RNA viruses (positive-strand) enhance the biogenesis of virus factories bound to the cytoplasmic membrane, and upon infection, the 6K2 protein-induced replication vesicles form chloroplast-6K2 complexes by targeting the chloroplast for replication [195]. The two host proteins Syp71 and Vap27-1 are associated with the chloroplast-bound 6K2 complex. Silencing of these factors showed that Syp71 but not Vap27-1 is essential for mediating the fusion of the virus-induced vesicles with chloroplasts during virus infection [196]. It was observed that two viral suppressors, carmovirus P38 and potyvirus HC-Pro require ethylene-inducible host transcription factor RAV2 to divert host defenses toward responses and block RNA silencing [197]. Furthermore, the Viral genome-linked protein (VPg) and HC-Pro suppressors interact with Decapping protein 2 (DCP2) and Exoribonuclease 4 (XRN4) respectively, both are key proteins of cytoplasmic 5′-3′ RNA decay gene-encoded pathway (5′ RDGs). The interaction results in the suppression of RNA silencing and promotes viral infection [198]. In DNA viruses, suppressors betaC1 (βC1) and AL2 proteins require host CaM for complete function. The βC1 expresses the CaM to repress the *RDR6* expression and stop the production of secondary siRNAs, while AL2 influences defense response genes through CaM and eventually blocks the RNA silencing [199,200].

4. Plant RNA Silencing Machinery against Fungi

Fungi are eukaryotic organisms, consisting of more than one million species and are considered as one of the most important groups of plant pathogens causing huge yield losses in various economic crops. Fungal pathogens penetrate the host through a natural opening, wound or through the specialized hyphal structures. The gene organization, cellular structures, and metabolic mechanisms of the fungus are similar to those of other higher eukaryotic organisms such as animals and plants. Beside the pathogenicity, certain fungal species also act as vectors for the transmission of different viruses. Similar to other eukaryotes, fungi also contain basic RNAi components to perform antiviral activities. The presence of RNAi components has been described in many fungal species [201–204], and these components have similar functions of antiviral activity like in higher eukaryotic organisms [205–208].

Plant and plant fungal pathogen share similar RNAi machinery, which on the one hand is used by the hosts to defend pathogens, while on the other hand, is utilized by the invading organism for growth, development, and pathogenesis. Fungal DCLs produce sRNAs that bind to the plant AGO1 proteins to hijack the RNAi machinery and silence host immunity (Figure 3). In the case of *Botrytis cinerea*, *dcl1 dcl2* double mutants show reduced virulence due to lack of plant immunity suppressing sRNAs produced by active *DCls* genes [209]. The *VmAGO2* of *Valsa mali* is critical for pathogen virulence which causes disease on apple leaves and twigs [210]. *Colletotrichum higginsianum* mutant study reveals that various DCLs, AGOs, and RDRs have no effect on the vegetative growth of fungus, however, Δ*dcl1*, Δ*dcl1*Δ*dcl2* double mutants, and Δ*ago1* mutants exhibit severe defects in conidia formation and morphology [211]. DCL double mutants of *Colletotrichum gloeosporioides* also lack conidiation and penetration capability to the host plant, leading to poor manipulation of the host immunity [212]. Conidia formation and the entrance of fungal pathogen to host cells are an important step in disease development and countering the host immunity. The above report described the importance of the RNAi components that are involved in the virulence nature of plant fungal pathogens.

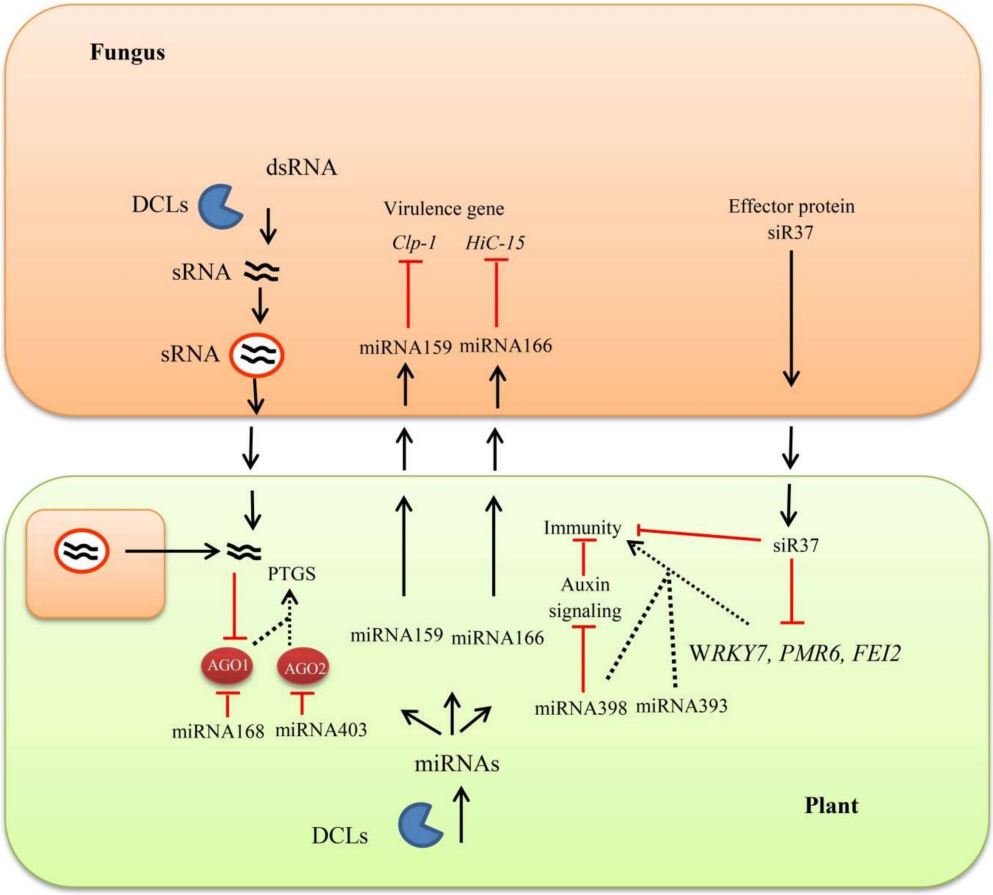

Figure 3. Role of RNAi in plant–fungal pathogen interaction. Plants activate biogenesis of various small RNAs or microRNAs (miRNAs) that enhance or inhibit certain signaling relating to the resistance or susceptibility against fungal pathogens. Some miRNAs are delivered to pathogens through cross-kingdom RNAi to perform silencing or inhibit virulence related genes of the interacting pathogens. The fungal pathogens also deliver certain effector proteins that hijack the RNAi components or suppress host defense related genes. Posttranscriptional gene silencing (PTGs), isotrichodermin C-15 hydroxylase (HiC-15), and $Ca2^{+}$-dependent cysteine protease (Clp-1).

The direct involvement and differential expression of the plant RNAi components showed contradictory results against fungal pathogens in different host plants. *Arabidopsis* mutants *dcl4*, *ago1*, *ago2*, *ago7*, *ago9*, *rdr1*, *rdr2*, *rdr6*, *sgs1*, *sgs2*, *sgs3*, and *nrpd1a* are more susceptible and display enhanced symptoms upon *Verticillium dahlia*, *Sclerotinia sclerotiorum*, and *Phytophthora species* infections [69,213–215]. However, silencing of *DCL1* enhances resistance to *Magnaporthe oryzae* and *S. sclerotiorum* in rice and *Arabidopsis*, respectively [69,216]. Such type of DCL1 interaction may be due to it being involved in the biogenesis of miRNA, and upon the infection of virulent fungus, the DCL1 reduces the accumulation of certain miRNAs, that induce the down-regulation of pathogen resistance genes [216]. It was previously found that the suppression of DCL1 resulted in the reduction of miRNA accumulation [217]. Similarly, knockout of AGO1 protein enhances resistance to *V. dahlia* and *Verticillium longisporum* [213,218]. The fungal virulence mechanism involves the AGO1 protein function through suppression of miR168 expression, as miRNA168 targets the RNA slicer enzyme of miRNAs pathways and the variation in expression of miRNA168-AGO1 plays an important role in plant–fungus interaction [218,219]. In tomato, the expression of two miRNAs suppresses the nucleotide-binding (NB) resistance (*R*) genes that have a function in plant immunity against *Fusarium oxysporum* and the potential resistance in the susceptible materials is insufficiently activated due to the action of these miRNAs [220]. Notably, miRNAs are non-coding sRNAs generated by DCLs from hairpin ssRNAs precursors, which target transcripts to regulate gene expression post-transcriptionally for cleavage or

translational repression [221]. Large numbers of miRNAs were reported to be functionally involved in fungal stress response. In *Brassica napus*, the *S. sclerotiorum* differentially expresses 68 miRNAs between the inoculated and un-inoculated leaves. A set of these miRNAs target nitric oxide- and reactive oxygen species-related and nucleotide-binding domain and leucine-rich repeat (NB-LRR) resistance (*R*) genes, and these genes are involved in plant defense response to various pathogens [214]. miR160a, miR396a, miR398b, miR482, miR1444, miR2118, and miR7695 are few examples of plants' microRNAs that are involved in gene regulation and immunity against fungal pathogens [222–226].

In plant–pathogen interaction, a diverse class of sRNAs contributes to host immunity through gene silencing but the pathogen-derived sRNAs also trigger pathogen virulence. Sometimes this influence can extend to other kingdoms and regulates gene silencing in the interacting organism; such type of interaction is called cross-kingdom RNAi. In cross-kingdom RNAi, the silencing signals translocate from both sides of the interacting organisms and perform gene silencing of the opponents. It was observed that *Arabidopsis* secreted extracellular sRNAs deliver vesicles at infection sites and are taken up by *Botrytis cinerea* cells [227]. Cotton plants induce biogenesis of two specific miRNAs upon *Verticillium dahliae* infection and export them to the fungal cells for silencing. Both miR159 and miR166 target different virulence-related genes of the fungal pathogens and confer disease resistance [228]. The secretion of these extracellular vesicles (EV) not only enhanced during biotic and abiotic stresses but also in response to hormonal treatment and contributes in plant innate immunity [229]. Using the cross-kingdom interaction, *B. cinerea* delivers sRNA effectors proteins into the host cells and down-regulates host RNAi and defense-related genes [230]. To protect the plant from pathogen sRNAs silencing, an alternative method of host-induced gene silencing (HIGS) is used to trigger plant immunity. In HIGS, exogenous artificial RNAi signals are expressed to target pathogen mRNAs for gene suppression and enhanced crop resistance. This approach is successively reported in plant–fungus interaction. In tobacco plants, interfering intron-containing hairpin RNA constructs transform to target chitin synthase (*chs*) gene, an important component of fungal cell wall chitin synthesis. RNA constructs silence the *chs* genes in transformed plants and provide efficient resistance against *S. sclerotiorum* [231]. Similarly, HIGS of an essential fungal chitin synthase gene (*Chs3b*) enhances resistance to *Fusarium* blight in wheat [232]. Suppression of *V. dahliae* virulence factors (*Ave1*, *Sge1*, and *NLP1*) through the HIGS approach reduces the susceptibility of *Arabidopsis* and tomato to *Verticillium* wilt [233]. Transgenic wheat plants cause severe hyphal cell wall defects in *Fusarium culmorum* via RNAi hairpin silencing construct against the β-1, 3-glucan synthase gene *FcGls1* and show enhanced resistance in leaf and spike [234]. Studies have revealed the similar role of HIGS in suppressing diseases caused by various groups of fungi [235–238].

5. Plant RNA Silencing Machinery against Bacteria

Plants and bacteria interact with each other in a variety of ways. The interactions may be useful, harmful or neutral. The deleterious interactions lead to the development of disease on the host plants, and the rapid expansion of the bacterial diseases make it difficult to control. Pathogenic bacteria restrict plant development by using their virulence factors delivered via multiple processes like the production of phytohormones, quorum sensing, siderophores, exopolysaccharides, and the Type III secretion system (T3SS) [239–244]. Plant primary defense mechanisms start after the recognition of bacterial translational or flagellum components. To counteract the pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) system, bacteria deliver various effectors into the host cells and alter the transcriptome and proteome of the cell to make it susceptible to the pathogen. The plant triggers the second defense layer in the form of effector-triggered immunity (ETI) after the recognition of pathogen effectors. Several endogenous siRNAs and miRNAs were reported to fine-tune PTI and ETI responses [245,246]. PTI and ETI mainly target the bacterial iron-related sigma factors to disturb bacterial iron metabolism, as high expression of these factors provides tolerance to bacteria against plant immunity [247].

The virulent *Pseudomonas syringae* contains type III secretion effector that suppresses the activity, stability, and biogenesis of pathogen-responsive miRNAs. *P. syringae* pv. *tomato* DC3000 *hrcC* mutant, a non-virulent form of bacteria due to lack of T3SS is unable to suppress PTI in wild-type *Arabidopsis* but enhances bacterial growth in the miRNA deficient *dcl1-9* and *hen1-1* mutants [248]. Similarly, *dcl1-9* and *hen1-1* mutants show similar symptoms of bacterial disease upon inoculation of the nonpathogenic *Escherichia coli* W3110 and *Pseudomonas fluorescens* Pf-5 strains [248]. In contrast, the *dcl1* and *hen1* mutants show enhanced defense against *Agrobacterium tumefaciens* induced gall formation and conversely, *rdr6* mutants are significantly susceptible to *A. tumefaciens* infection [249], revealing that a successful infection needs a strong anti-silencing mechanism to inhibit specific siRNA synthesis. It was reported that DCL1, DCL4, AGO7, HYL1, HEN1, Hasty1 (HST1), RDR6, and Pol IV, are involved in the biogenesis of long siRNAs (lsiRNA-1), which is also induced by effector *avrRpt2* carrying bacterial infection. In *Arabidopsis*, AtlsiRNA-1 induction targets RNA binding domain abundant in apicomplexans (*AtRAP*) mRNA and promotes down-regulation of the targeted mRNA (Figure 4). This down-regulation of *AtRAP* enhances resistance to both virulent and avirulent strains of bacteria as observed in *AtRAP* knockout mutant. The AtlsiRNA-1 down-regulates the target mRNA via decapping and XRN4-mediated degradation [250].

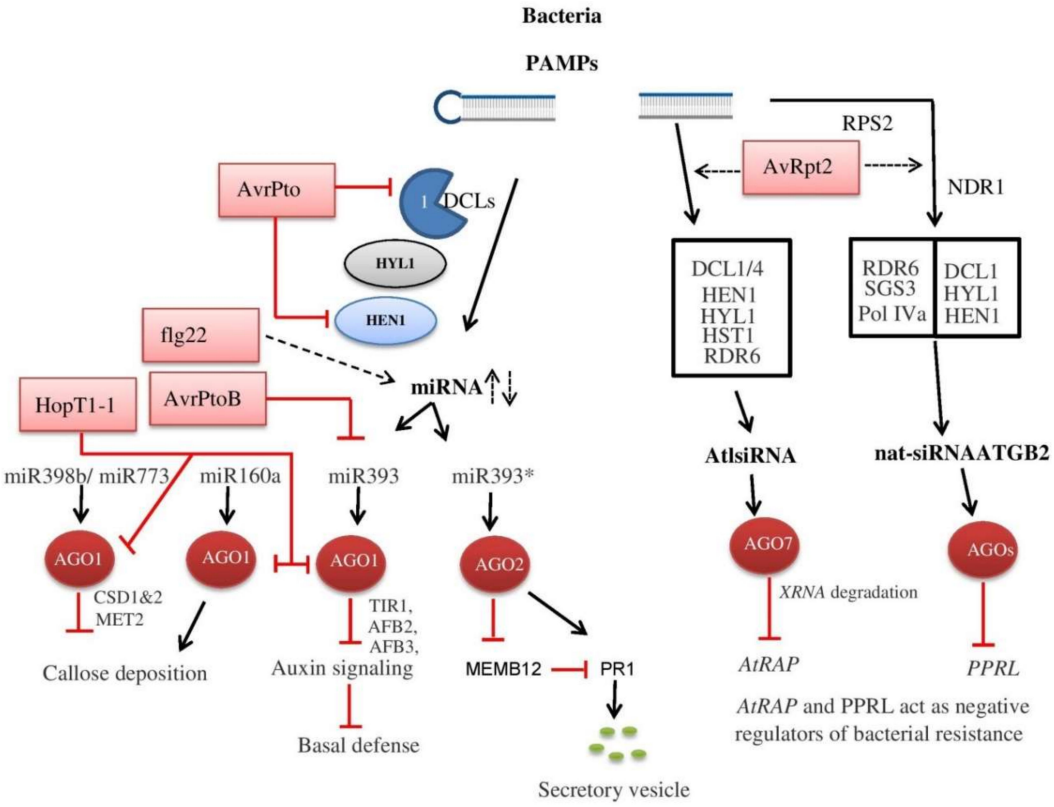

Figure 4. Role of RNAi in plant–bacterial interaction. Upon bacterial infection, plants detect pathogen-associated molecular patterns (PAMP) and control the accumulation of various siRNA or miRNAs through RNAi machinery. These sRNAs either enhance the defense related signals and resistance genes or silence certain genes that negatively regulate plant immunity. In response to the PAMP-triggered immunity (PTI) system, bacteria produce certain effectors that suppress host RNAi mechanism (pink box). Hasty1 (HSTY), Resistance protein 2 (RPS2), Non-race specific disease resistance protein (NDR1), Pentatricopeptide repeats protein-like (PPRL), RNA binding domain abundant in apicomplexans (RAP), MEMB12 (Membrin), Pathogenesis-related protein (PR1), Transport inhibitor response 1 (TIR 1), Auxin signaling F-Box proteins 2 and 3 (AFB2&3), Copper superoxide dismutases 1 and 2 (CSD1&2), Methyltransferase (MET).

Another type-III bacterial effectors (*HopT1-1*) interacts with AGO1 through conserved Glycine/Tryptophane (GW) motif and suppresses the AGO1-dependent miRNA pathway to promote pathogenicity in *Arabidopsis*. AGO1-dependent miRNA positively regulates PAMPs-induced callose deposition and bacterial resistance. The bacterial flagellin-derived peptide flg22 differentially expresses certain AGO1 dependent miRNAs, and the overexpression of the selected miRNAs revealed that miR160a positively while miR398b and miR773 negatively regulate callose deposition and disease resistance to *P. syringae* [251]. The flg22 also triggers the AGO1 binding to promote transcription of innate immune and chitin responsive genes [252]. The derived-peptide induced miRNAs (miR393) down-regulate mRNA for F-box auxin receptors and suppress auxin signaling to enhance basal defense against bacteria [253]. The miR393 pair was identified to function in antibacterial immunity through two distinct AGOs proteins. AGO1 associated miR393 and AGO2 associated miR393* contribute to PTI and ETI, respectively [254]. During plant growth and development, the miR390 participates in auxin signaling through the production of *trans*-acting siRNAs (tasiRNAs) from *T*rans acting siRNA *3* (*TAS3*) transcripts to regulate auxin responsive factor (*ARF*) genes by DCL1 processing and AGO7 as an associated partner [255]. It is unclear how the two contrasting, miR393 and miR390-*TAS3-ARF* pathways of auxin signaling correlate with each other during bacterial infection. However, a small class of Auxin response factors (ARF) also involves in repression of downstream auxin-regulated genes [256], and it might be possible that miR393 activate these factors to inhibit miR390-*TAS3-ARF* pathway. AGO4 is the important component of RdDM pathway that works independently in regulating resistance to *P. syringae* pv. *tomato* DC3000. Loss of function in RdDM pathway components, upstream or downstream of AGO4 does not facilitate resistance to *P. syringae* [257]. Therefore, after bacterial infection, flg22 down-regulates AGO4 to repress RdDM pathway and inhibit TGS [258].

RDR6 is a key RNA silencing factor studied in plants against bacterial pathogens. RDR6 is involved in the biogenesis of bacterial-induced lsiRNA-1 and natural antisense transcript (NAT)-associated siRNAs (nat-siRNA, e.g., nat-siRNAATGB2), and knockout *rdr6* mutants are highly susceptible to *P. syringae* pv. *tomato*, *A. tumefaciens*, and *Xanthomonas oryzae* pv. *oryzae* [249,250,259,260]. Studies on various plant species showed that several miRNAs target ETI associates with NB-LRR encoded *R* genes and generate secondary siRNAs or phased siRNAs (phasiRNA) in an RDR6-dependent manner [261–264]. Three highly abundant 22 nt miRNA families were identified in *Medicago* that target at least 114 conserved domains of defense-related NB-LRR encoded *R* genes to enhance production of secondary siRNAs or phasiRNAs [261]. *Arabidopsis* miR825* and tomato miR482 target the NB-LRR type *R* genes and their expression negatively correlate with target genes. Upon bacterial infection, these miRNAs are suppressed to activate the target defense-related genes [263,265]. In *Arabidopsis*, *RDR6,* and miR472 suppress both basal and RPS5-mediated resistance to *Pto* DC3000 through controlling coiled-coil nucleotide-binding leucine-rich-repeats (*CNLs*) family genes [266]. Comparing the role of RNAi mechanisms against different pathogens, it is observed that lack of function of one protein can be substituted by other during viral and fungal infections, while, in case of bacterial infection, there is a lack of information about the effect of down-regulation or suppression of the particular RNAi component on the function and expression of other members of this pathway. Table 2 describes the basic functions of RNAi pathway key components involved in the counter defense of plant pathogens.

Table 2. Diversity of RNA interference (RNAi) pathway essential components involved in plant immunity.

Proteins	Components	Functions	References
Dicer-like protein	DCL1	Biogenesis of 21 nt siRNAs, miRNAs, nat-siRNA and lsiRNA, involved in PAMP-triggered immunity	[69,216,251,253,267]
	DCL2	Production of 22 nt siRNAs and stimulates transitivity	[84,167,268]
	DCL3	Biogenesis of 24 nt siRNA and hc-siRNA, involved in chromatin modification and transcriptional silencing	[29,30,88]
	DCL4	Biogenesis of 21 nt siRNAs and processed ta-siRNAs	[28,69,89,269]
Argonaute protein	AGO1	Major AGO protein that associates with vsiRNAs, involved in miRNA-directed gene silencing and posttranscriptional gene silencing	[35,116,251,270]
	AGO2	miRNA-directed gene silencing, repress translation, and played role in immune responses	[84,92,254,271]
	AGO4	Bind 24 nt siRNAs to form RdDM complex, involved in DNA methylation and transcriptional gene silencing	[129,135,257,272]
	AGO5	Bind 21-, 22-, and 24 nt siRNAs, involved in posttranscriptional gene silencing and systemic resistance	[119,273]
	AGO7	Required for generation of lsiRNAs and contributed to effector-triggered immunity	[250,251]
RNA-dependent RNA polymerase	RDR1	Amplification of siRNA and production of dsRNA, limit systemic infection	[69,89,136,152]
	RDR2	Production of secondary dsRNA through RdDM pathway and involved in regulation of transposons	[61,62,89]
	RDR6	Biogenesis of ta-siRNAs, nat-siRNAs, and secondary siRNA, involved in cell to cell silencing signal and posttranscriptional gene silencing	[250,259,274]
Double-stranded RNA binding proteins	DRB1 (HYL1)DRB2-DRB4	Interact with DCls for the efficient production miRNA, tasiRNAs, siRNA, nat-siRNA and lsiRNA	[102–104,106]
HUA enhancer 1	HEN1	Biogenesis of lsiRNA and nat-siRNA, stabilized and methylates all endogenous sRNAs	[97,259,275]
Heat shock protein	HSP70-90	Role in RISC formation and siRNA loading	[42,43,276]
Cofactors	SGS	Stabilized the RISC-cleavage and facilitated RDR activity	[50,277]
	SDE	Accumulation of tasiRNAs and facilitated RDR activity in conversion of ssRNAs in to dsRNA	[154,155]

Small interfering RNAs (siRNAs), microRNAs (miRNAs), Natural antisense transcript-derived siRNAs (nat-siRNAs), Heterochromatic siRNAs (hcsiRNAs), Virus-derived siRNAs (vsiRNAs), Long siRNAs (lsiRNAs), Double-stranded RNAs (dsRNA), Small RNAs (sRNAs), Single-stranded RNAs (ssRNAs), *Trans*-acting siRNAs (tasiRNAs), RNA-directed DNA methylation (RdDM), Suppressor of gene silencing (SGS), Silencing defectives (SDE), RNA-induced silencing complexes (RISCs).

6. Conclusions

In recent years, highly advanced technologies and molecular works have unfolded many pathogen-induced plant defense responses and strategies during stress conditions. However, the interaction of plants with various virulent pathogens is so complicated that the exact mechanism of plant natural immunity or resistance remains elusive. Although researchers have identified a large number of resistance genes in different crops, the question still remains of how to classify the role of specific genes in a particular plant–pathogen interaction. In this review, we summarized the RNAi mechanism, a common plant endogenous defense machinery, against all kind of pathogens. We discussed the important components and function of each protein member in silencing of invading nucleic acid based on previously published reports. The three main components of RNAi machinery and their cofactors collaborate with each other against the foreign RNA/DNA or suppressors of the invading pathogens. This mechanism also holds certain limitation regarding their activation and function. Pathogen-induced differential expression of some components results in the contradictory immunity responses raises questions on silencing pathways. Second, the function of many DCLs, AGOs, and RDRs are still largely unknown, particularly the antibacterial activities of RDR6-dependent miRNA target defense genes. One of the important points highlighted in this review is that RDRγ

protein function is still not fully studied and this group needs more research to fully understand the complete mechanism.

In conclusion, the plant RNAi mechanism provides basic information to develop naturally immune crops against the virulent pathogens. Further research needs to understand the exact mechanism and cooperative strategies of RNAi pathway components that will hopefully resolve the complex plant defense system against various pathogens.

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