Int.J.Curr.Microbiol.App.Sci (2020) *9*(9): 3114-3125

EXCELLENT PUBLISHERS

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 9 (2020)**
Journal homepage: http://www.ijcmas.com

Review Article https://doi.org/10.20546/ijcmas.2020.909.384

RNA Interference (RNAi) and Response of Plant Cells to Double Stranded RNA (dsRNA)

Gowthamkumar Routhu*, Munmi Borah, P. D. Nath and Bisakha Deb

Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam (785013), India

**Corresponding author*

Keywords

RNAi, dsRNA, endogenous RNA, Exogenous RNA, siRNA

Article Info

Accepted:
20 August 2020
Available Online:
10 September 2020

ABSTRACT

RNA interference (RNAi) mechanism is a natural process occurring in both plants and animals which silence the specific genes before being translated. RNAi is the key biological process in plants and has many different forms including transcriptional gene silencing (TGS) and post transcriptional gene silencing (PTGS). Plants can uptake and process externally applied double stranded RNAs, hairpin RNAs and small interfering RNAs prepared to silence genes of viruses, fungi and insects. The exogenously applied small RNAs move locally and systematically and induce RNAi mediated resistance. Understanding the plant response and processing of small RNAs could result in development of novel biotechnological approaches. In this review we analyze the biogenesis and function of endogenous set of small RNAs, exogenously applied small interfering RNAs and their response in plant cells.

Introduction

The phenomenon of RNA interference was first discovered in a nematode worm *Caenorhaditis elegans*, where double stranded RNA (dsRNA) bring about specific gene degradation of mRNA which results in gene silencing. RNAi mechanism in plants gives different types of small RNAs (sRNAs) including siRNAs, miRNAs, ta (*trans*-acting)-siRNAs, and pha (phased)-siRNAs. siRNAs are generated from various Double stranded RNA molecules(dsRNAs) i.e., dsRNA derived from RNA virus replication, dsRNAs synthesized by plant endogenous RNA-dependent RNA polymerase, dsRNAs of natural sense and antisense transcripts, and single-stranded RNAs that form hairpin-loop secondary structures (Baulcombe 2004; Voinnet *et al.,* 1999; Rosa *et al.,* 2018). These dsRNA molecules are recognized and cleaved by Dicer like proteins present in the cell to generate siRNAs. After cleaving of dsRNA into siRNA, the siRNA duplex molecules move into RNA-induced silencing complex (RISC), where Argonaute (AGO) protein plays a major role. The RISC complex cleaves the siRNA duplex, where one strand retains and serves as guide strand. The guide strand serves as a recognition molecule to identify the cognate mRNA molecule which is translating into protein. The guide strand

recognizes the mRNA strand by Watson-Crick base pairing, leading to RISC cleavage/ slicing of RNA target. Plants encode multiple AGO proteins, the detailed description of evolution and function of different AGOs can be found in the review of Zhang *et al.,* 2015. Among all the Argonaute proteins AGO2 has been reported to be involve in siRNA-related antiviral, antibacterial and anti-stress defenses (Jaubert *et al.,* 2011). AGO protein contain three major domains like PAZ, MID, and PIWI domains. After slicing the small RNA target duplex is separated by variable domain (ND) present at the N-terminal region of AGO proteins. The PAZ domain, which is close to ND bind the 3' end of small RNAs (guide RNA) (Lingel *et al.,* 2004). MID and PIWI domains are present in C-terminal domain. The junction of these two domains contains a pocket which anchors the 5' end of the guide RNA. The siRNA- target duplex loaded AGO protein cleaves the target RNA by PIWI domain, which contains a RNase-H-like structure and a conserved catalytic site (Parker 2010). By the action of RNase-H the target RNA is cleaved and degraded. By this functional mechanism the target gene or protein is degraded without undergoing to translation thereby disrupting the central dogma of life.

Endogenous small RNAs

Small RNAs or micro RNAs control the gene expression mechanism by silencing the target mRNAs without encoding into protein. In plants small RNAs perform many functions such as to control expression of genes encoding transcription factors, stress response proteins and other proteins which are involved in the growth, development and physiology of plants (Rogers and Chen, 2013).Small RNAs are mainly classified into micro RNAs (miRNAs) and small interfering RNAs (siRNAs) (Parent *et al.,* 2012). Both miRNA and siRNA are derived from double stranded RNA molecules. miRNAs are derived from the hairpin loop precursors from the single double stranded RNA molecule (dsRNA) whereas siRNAs are derived from the double stranded duplexes of the two RNA molecules. The first miRNA *lin-4* was discovered in *Caenorhabditis elegans* in the year 1993 by Victor Ambros and his colleagues.

miRNA pathway

In plants miRNA biogenesis begins in nucleus with the generation of long (>70nt) bulged hairpin RNA known as primary microRNA (pri-miRNA). In plants miRNAs are derived from the non coding regions (Introns) and few of them are located in 5' UTR or 3' UTR regions of a nuclear gene and animal miRNAs are encoded from the introns of protein coding genes. Plant miRNAs are transcribed by RNA Pol II which produces capped and poly-adenylated long primary miRNA transcripts (pri-miRNAs), these are then processed into precursor miRNA (pre-miRNA) of about 80-500 nucleotides in length, by an enzyme, DICER-LIKE 1 (DCL 1), with RNase III activity (Kurihara *et al.,* 2006). A part of pri-miRNA forms hairpin loop like structure by folding which is cropped by DCL 1 complexes. The DCL 1 enzyme complexes cut the pri-miRNAs at a distance of 16-17bp from the miRNA duplex in the nucleus (Zhu *et al.,* 2013). The conversion of pri-miRNA to pre-miRNA requires various proteins like double-stranded RNA (dsRNA)-binding protein Hyponastic Leaves1 (HYL1) and C2H2 zinc finger protein SERRATE (SE) along with other factors like CBC, DDL, and TOUGH (Yang *et al.,* 2006; Parry *et al.,* 2007). These proteins are present near the nuclear processing centers called D-bodies or SmD3-/SmB-bodies (Kurihara *et al.,* 2006). Pre-miRNA is unstable in nucleus and processed into miRNA-miRNA duplex by DCL1 and

stabilized by S-adenosyl methionine dependent methyltransferase HUA ENHANCER 1 (HEN 1), which methylates all plant silencing RNAs. The mature miRNA duplex are then exported to cytoplasm by the action of HASTY protein (an ortholog of exportin-5 protein) (Bollman *et al.,* 2003). According to their position on the hairpin-shaped precursor the miRNA and miRNA* are named as miRNA-5p and miRNA-3p respectively (Kozomara *et al.,* 2014). The produced miRNA-5p or miRNA-3p are methylated by HEN1, which protects from being degraded by small RNA degrading nuclease (SDN) class of nucleases (Ramachandran and Chen 2008). Finally the functional miRNA will be incorporated into Argonaute protein containing RNA Induced Silencing Complex (RISC). Plant miRNAs associate with AGO1 protein to induce pot-transcriptional gene silencing (PTGS) resulting in RNA silencing or translation inhibition (Li *et al.,* 2013).

Trans-acting siRNA pathway

In plants, a group of microRNAs (miRNAs) induce the production of class of small interfering RNAs (siRNAs) which lead to gene silencing are called trans-acting siRNA (ta-siRNA) (Jacobs *et al.,* 2016). The pathway for trans-acting RNA is a combination of both miRNA and siRNA pathway but have a different mechanism. Like miRNA, ta-siRNAs are 21 nucleotides long and are derived from the intrgenic regions of the nuclear gene. In ta-si RNA biogenesis, a single stranded RNA transcript is first cleaved by AGO1 protein directed by miRNA. The cleaved product is converted into long dsRNA molecule by RDR6, which is then processed by DCL4 into 21-nt siRNAs with 21 nucleotides phasing starting from the mi RNA cleavage site (Xie *et al.,* 2005). For the production of ta-siRNA in *Arabidopsis* suggests that 22 nucleotides size of miRNA is sufficient to induce ta-siRNAs, whereas 21-nt of miRNA are not (Chen *et al.,* 2010). Like miRNAs methylation, the ta-si RNA molecules are methylated by HEN1 and interact with AGO1 or AGO7 to direct the degradation of target mRNA. In *Arabidosis,*ta-siRNA affect the auxin response involved in phase transition from initial juvenile stage to reproductive stages (Fahlgren *et al.,* 2006). A large number of ta-siRNA like siRNAs are collectively known as phased siRNAs or phasiRNAs. PhasiRNAs have been identified in *Arabidosis*, tomato (Shivprasad *et al.,* 2012), tobacco (Li *et al.,* 2012), rice (Song *et al*, 2012) and in many other crops. RNA sequence from protein-coding genes (Exons) and non-coding genes (introns), transposons and repetitive DNA can serve as a template for phasiRNA production. PhasiRNAs are derived from the Leucine rich Repeat (NB-LRR) disease resistance genes and play a major role in controlling the stress proteins and defense related genes (Fei *et al.,* 2013).

Exogenous small RNAs

Doule stranded RNA (dsRNA) act as a signal molecule to induce RNAi in plants and higher organisms. When a dsRNA molecule corresponding to endogenous mRNA is introduced into the cell of the organisms, the cognate mRNA is silenced. This phenomenon was discovered in *Caenorhabditis elegans* where dsRNA corresponding to specific gene injected in to worm the corresponding gene was disappeared (Fire *et al.,* 1998). A dsRNA molecule of 21nt long could trigger RNAi in human cells without inducing interferon response was reported by Elbashir *et al.,* In a study conducted by Elbashir *et al.,* synthetic siRNAs are constructed to mimic dsRNA RNAi trigger in eukaryotic cells. Plants can uptake synthetic dsRNA or siRNAs when exogenously applied and also capable of entering in to vascular bundles and distant

cells(Bennett *et al.,* 2020). SiRNAs are synthesized commercially using solid phase synthesis. *In vitro* transcription can also e used to synthesize siRNAs or short hairpins. Exogenously applied siRNA can induce silencing as that of miRNA pathway, the difference is that exogenously applied dsRNA already mimic the product of Dicer. siRNA sequences are designed bas on Watson-Crick base pairing complemenatarity to the target mRNA. As a result siRNA-mediated RNAi result in cleavage of target mRNA by the slicer activity of Ago2 protein. Finally the target mRNA is degraded an cleaved. Short hairpin RNAs (shRNAs) are small RNAs which are structurally similar to siRNAs an miRNAs. Like siRNAs, the guide strand and the passenger strand are perfectly complementary to each other. shRNAs are designed to function like as that of both miRNA and siRNA. shRNAs can be synthesized artificially and can be transfected into cell like siRNAs. shRNAs can be expressed from plasmid DNA or viral vectors in the eukaryotic cells. The small RNAs can e applied onto plant leaves by mechanical inoculation, spray application, loading on nano clay sheets, materials that promote RNA adhesion, spreading by pipettes or brushes and root soaking (Dubrovina and Kiselev, 2019).

Induction of dsRNA in plants transgenically

Double stranded RNA (dsRNA) derived from transgenes of sense and antisense RNA homologus to coding gene can induce RNA interference (RNAi) mechanism in plants. Plants engineered with dsRNA gives specific and robust resistance against plant viruses through RNAi. Transgene based RNAi approach was firstly used in plant virus disease control. hpRNAs have been widely used to silence the target genes and viral RNAs in plants (Watson *et al.,* 2005). For the host delivery of RNAi, plants are genetically modified to express dRNA molecules with sequence derived from the target gene. RNAi constructs are made by cloning a fragment of target gene coding sequence in sense and anti sense orientation, separated by intron/spacer region, under the control of a constitutive or tissue-specific promoter. Upon transcription, sense and antisense strands of the target gene complement each other and form an intron spliced hairpin RNA of which a significant portion is dsRNA (Waterhouse and Helliwell, 2003; Dutta *et al.,* 2015). Most researchers have constructed stable transgenic plants in which the transgene constructs generate double-stranded (ds) RNA, either directly or indirectly. The dsRNA is then processed to siRNA in vivo by Dicer which is an endogenous enzyme present inside the cell. Viruses can also be used to deliver the silencer sequences using a transient silencing protocol. Both of these basic protocols use *Agrobacterium tumefaciens* to deliver transgene constructs into plant hosts (Yin *et al.,* 2005). Compared to the application of chemicals, transgenic plants are cost effective and a promising tool. While there are legislative limitations on cultivation of transgenics in many countries, the information on consequences of genome modification are insufficient and raises public concerns on safety of genetically modified organisms (Dubrovina and Kiselev, 2019). Virus resistant plants are generated by RNA silencing mechanism by expressing virus coat protein genes. Transgenic papaya plants were generated against *papaya ring spot virus*, coat protein gene isolated from *papaya ring spot virus* was cloned into a binary vector with 35S promoter and transformed into somatic embryos of papaya cultivar by microrojectile bombardment. The transformed transgenic lines of potato showed resistance against *papaya ring spot virus* due to RNA mediated interference (Kerbundit *et al.,* 2007). Transgenic plants of potato expressing

hpRNA construct targeted against *plasma membrane-localized Syntaxinrelated 1* (*SYR1*) showed enhanced resistance to the oomycete pathogen *Phytophthora infestans* (Eschen-Lippold *et al.,* 2012).

Methods to induce RNAi in plants

An important aspect to induce RNAi in plants is to deliver the molecules of silence inducing dsRNA or hpRNA. RNA can e delivered by transforming plants with transgenes that encode hpRNAs or viral RNAs. The most commonly used methods for delivery of dsRNA in plants cells are agroinfiltration, microbombardment and virus induced gene silencing (VIGS).

Agroinfiltration

Agroinoculation or Agroinfiltration is the process of delivering active DNA molecules by using transformed *Agrobacterium* cells into plant cells. When *Agroacterium* is injected into the cell it transfers the T-DNA plasmid and integrates in the nucleus of the host cell. The genes of th T-DNA express when they are introduced into the cell. When the leaves are infiltrated with *Agrobacterium* culture containing T-DNA region with a transgene that encodes a endogenous gene will trigger the RNAi mechanism. The infiltration of hairpin constructs are very effective, because the dsRNA is directly processed into siRNA (Johansen *et al.,* 2001). Leaf infiltration of *Agrobacterium* is the popular method for transient gene expression in *Nicotiana benthamiana* (Goodin *et al.,* 2008). Agroinfiltration method has been successfully employed in *Arabidosis* (Wroblewski *et al.,* 2005) and in many other crops like Tobacco (Yang *et al.,* 2000), Tomato (Orzarez *et al.,* 2006), Grape vine (Santo-Rosa *et al.,* 2008) etc. Agroinfiltration provide a reliable and convenient way in achieving level of gene expression like inducing resistance to crown gall disease in apple (Dunoyer *et al.,* 2006), OsGEN-L-green fluorescent (GFP) fusion protein in rice (Moritoh *et al.,* 2005) etc.

Microbombardment

In this method the DNA is transferred into the nucleus of the cells by nuclear bombardment. Plant tissues are bombarded with gold or tungsten particles that have been coated with DNA is a routinely approach for studying the gene expression in monocotyledonous plants including rice (Christou, 1997). Cells bombarded with particles containing dsRNA, siRNA or hpRNA trigger the RNA silencing pathway in plants. A repressor of GUS protein was silenced in transgenic tobacco (*Nicotiana tobaccum*) plant using micro bombardment of dsRNA was reported by Klahre *et al.,* 2002.

Virus Induced Gene Silencing (VIGS)

Virus Induced gene silencing is a reverse genetic tool, which uses viral vectors to produce dsRNA which induce RNA mediated gene silencing in plants. VIGS exploits the innate plant defense system of Post Transcriptional Gene silencing against intracellular viral replication and proliferation (Lange *et al.,* 2013). Most of the plant viruses contain RNA as their genetic material. When a virus infects the cell the RNA is released upon the disassembly of Coat protein. The RNA is then replicated by the virus encoded RNA dependent RNA polymerase enzyme to produce sense and anti-sense strand RNA i.e dsRNA. The viruses which are capable to produce the dsRNA trigger the RNAi response in plant cells. The viral genomes are modified such that viral RNAs are generated by invitro transcription and are placed in the left and right borders of T-DNA plasmid region under the control of CaMV 35S promoter (Liu *et al.,* 2002). The construct is

delivered into plant by Agroinfiltration and can produce viral infection triggering the RNAi response. Many of th viral vectors are derived from the single stranded RNA viruses like *Potato virus X* (PVX), *Tobacco mosaic virus* (TMV) *and Tobacco rattle virus* (TRV) (Purkayastha and Dasgupta 2009). As compared to RNA viruses, DNA viruses have not been commonly used as expression vectors because of their size constraints in their movement (Kjemtrup *et al.,* 1998). Expression vector derived from the Turnip Yellow Mosaic virus (TYMV) has ability to induce VIGS in *Arabiopsis thaliana* (Pflieger *et al.,* 2008). Several monocot plants are successfully targeted by VIGS like BSMV and BMV in *Hordeum vulgare* (Holzberg 2002), BMV and RTBV in *Oryza sativa* (Purkayastha *et al.,* 2010), BSMV in *Triticum aestivum* (Scofield 2005) and BMV in *Zea mays*(Ding at al., 2006).

Induction of dsRNA in plants non-transgenically (RNA based vaccine)

RNA silencing is thought to play an important role in the protection against invading nucleic acids in plants towards its application as a result (Bartel 2004; Baulcombe 2004). External application of invitro synthesized or bacterially produced dsRNA showed resistance against viruses, invading pathogens and insect pests. The exogenously applied dsRNA spread locally and systematically to phloem bundles and to distant cells and induce RNA interference-mediated plant pathogen resistance. Many studies reveal that exogenously applied RNA molecules are capable of affecting the mRNA of target genes which leads to mRNA degradation in plant pathogens. For the topical application of dsRNA in a non-transgenic manner, dsRNA are expressed either by using invitro or invivo production systems with appropriate expression vectors (Robinson *et al.,* 2014). The vector elements required for RNA transcription are T7 or T3 DNA-dependent RNA polymerase (DdRp) promoters and a multiple cloning sites for insertion of sequence to be transcribed. For the production of dsRNAs, two invitro systems are available to induce virus resistance. Studies conducted by Tenllado and Diaz-Riaz 2001 used invitro single stranded RNA (ssRNA) transcription system for the production of dsRNA for topical application. In the *invitro* transcription system, they have transcribed by using two DNA plasmids each encoding a sense and anti-sense complementary DNA (cDNA) sequence from the respective virus was transcribed into ssRNA by T7 DdRp and the respective sense and antisense ssRNA subsequently annealed. In the second invitro system, *Pseudomonas syringae* dsRNA bacteriophage phi 6 (ϕ6) RNA dependent RNA polymerase (RDR) is used for the generation of full length dsRNA from ssRNA templates (Aalto *et al.,* 2007). In this system the insert is transcribed by T7 DdRp to produce the ssRNA template strand for the RDR of ϕ6 that subsequently uses the transcribed ssRNA to generate the complementary strand within single plasmid vector in a *E.coli* bacteria. For the large production of dsRNAs invivo expression systems were used which uses E. coli strain HT115 (DE3). A study conducted by the Timmons *et al.,* 2001to silence the endogenous gene of *Caenorhabditis elegans,* they have used E. coli strain HT115 which harbours the pro-phage λDE3 encoding the Isopropyl β-D- 1-thiogalactopyranoside (IPTG) inducible T7 polymerase gene for dsRNA transcription. IPTG acts as inducing molecule to activate the transcription and to produce dsRNA. The dsRNA derived by this method are very efficient and can be produced in large amounts (Gan *et al.,* 2010). dsRNA sequences derived from the viral sequences and introduced into tobacco plant by agro-inoculation showed resistance against three virus groups namely tobamovirus, potyvirus

and alfamovirus group (Tenllado and Díaz-Ruíz, 2001). Some more examples for non-transgenic based RNAi approach are listed below (Table 1):

Table.1 Report on Induction of dsRNA to plants non-transgenically (RNA based vaccine)

Crop	Virus	Virus derived region	Observation Recorded	Reference
N. tabacum* cv.Xanthi, *C. chinense	**PMMoV**	Virus replicase gene (977 bp)	Significant reduction of PMMoV virus in tobacco plant	Tenllado and Diaz-Ruiz, 2001
Bhut Jolokia	Cucumber Mosaic Virus	CMV-2b	Disease incidence ranged from 0 to 29% in dsRNA treated plants in contrast to 55 to 92% when only CMV was applied	Borah *et al.,* 2018
Sesbania grandiflora	Sesbania mosaic virus (SeMV)	Coat protein(CP) and (Movement Protein)	dsRNA of CP and MP conferred 53% and 64% resistance respectively	(Konakalla *et al.,* 2019)
Tobacco	Tobacco Mosaic Virus(TMV)	p126 and CP	dsRNA of p126 and Cp conferred 65% and 50% resistance respectively.	(Konakalla *et al.,* 2016)
Cucurbits-Cucumber Watermelon and Squash	Zucchini Yellow Mosaic Virus (ZYMV)	Helper Component Proteinase (HC-Pro)	exogenous dsRNA application in cucumber, watermelon and squash plants, dsRNA HC-Pro conferred resistance of 82%, 50% and 18%, and dsRNA CP molecules of 70%, 43% and 16%, respectively.	Kaldis *et al.,* 2018

Barriers in foliar uptake of dsRNA

Double stranded RNA (dsRNA) when sprayed on plants produce an elicit response to induce RNA interference RNAi. Foliar application of dsRNA should be in sufficient number to reach the target cells of the plant. The dsRNA sprayed must travel the surface of the leaf, through cuticle, to cross through the cuticle, cell wall and plasma membrane to gain entry into cell's RNAi machinery. Once it enters into cell, the dsRNA move to adjacent cells through plasmodesmata and to distal cells by phloem vessels. Small dsRNA generated in the cell's machinery can move through symplastically into phloem vessels and to distant cells. There are many barriers in the efficient delivery of dsRNA to the target cells (Bennett *et al.,* 2020).

Cuticle acting as a barrier

Cuticle layer composed of lipids and hydrocarbons covering the epidermis of leaves, young shoots and fruit. Cuticle is known to impede the absorption of exogenous

water and solutes The cuticular properties makes it to resistant to dsRNA absorption given the water solubility of dsRNA. To test this fluorescently labeled (Cy3) 21 base pair (bp) siRNA was applied on the adaxial surface of Palmer Amarnath (*Amaranthus palmeri*) with 0.5% of spreading surfactant Silwet L-77. Treated leaf samples were collected after 4hrs of application and observed under fluorescence microscopy. It was observed that most of the applied dsRNA was found on the adaxial surface itself. The fact that Cy3-SiRNA remained on the leaf surface due to its high molecular size and relatively water soluble molecule (Schreiber, 2005).

To increase the cuticle penetration of siRNA and to achieve RNAi phenotypes abrasion, high pressure spraying and abaxial stomotal flooding has been suggested. Spraying particles like celite, alumina of sizes >2 microns with or after siRNA application at pressures <700 kPa resulted in improved cuticle penetration of the siRNA and resulted in robust visual RNAi phenotypes (Huang et al, 2018).

Nuclease stability as a Barrier

In vivo nuclease degradation is known to impact the efficiency of applied siRNA to elicit an RNAi response in mammalian systems (Behlke, 2008). Very less information is known about the impact of nucleases on delivery efficiency of siRNA in plants. It was suggested that nuclease degradation could impact the ability of foliar applied siRNA to gain access to plant cell (Pérez-Amador *et al.,* 2000). An experiment was conducted to investigate the impact of nucleases on siRNA activity where 22bp siRNA was applied by syringe infiltration to an leaf of *N. benthamiana*. The 22 bp siRNA was infiltrated in the presence or absence of nuclease inhibitor or the cationic polymer polybrene. Leaf tissue samples were collected from infiltration site at 0, 1, 2, 4, 6, 8, and 24h post application. The results were analyzed using anionic exchange HPLC. It was observed that when siRNA applied in absence of nuclease inhibitor or polybrene was not detectable at 6 h post application whereas siRNA applied with a nuclease inhibitor or polybrene which binds dsRNA still detectable at 24h application (Schreiber, 2005). The results suggest that nucleases act as a barrier to efficient dsRNA delivery.

Response activated by dsRNA in Plants

Plants have evolved with a variety of immune systems like Pathogen Associated Molecular Patterns (PAMPs)- triggered immunity (PTI), Effector Triggered Immunity (ETI). When a pathogen infects the plant the immune systems are activated and employ resistance (R) proteins to activate ETI. The pathogen avr genes react with R proteins which is called R mediated gene resistance leading to Hyperensitive response (HR). the first R gene conferring resistance was identified as tobacco N gene in *Tobacco Mosaic virus* and Avr gene was identified in the replicase gene (Whitham *et al.,* 1994). Unlike fungal and bacterial pathogens viral genomes replicate within host leads to RNA silencing pathways and play a critical role in anti-viral defense. During the replication of the RNA/DNA viruses dsRNA is the common molecule and act as an intermediate. Upon the recognition of dsRNA molecule in plant RNA silencing and Pathogen Triggered immunity gets activated in the plant (Niehl *et al.,* 2018). The other responses that are induced by viruses and dsRNA in plants include the production of reactive oxygen species (ROS), induction of hormone signaling, activation of mitogen activated protein kinases and induction of defense gene expression (Nicaise and Candresse, 2017). The anti-viral immune response is mediated by Leucine-Rich-Repeat

receptor like kinase (LRR RLK) NIK1. Upon activation of NIK1 during infection, a signaling cascade leading to down regulation of gen associated with translational machinery and eventually the down regulation of viral and hosts mRNA translation, is induced.

References

Aalto AP, Sarin LP, van Dijk AA, Saarma M, Poranen MM, Arumae U, Bamford DH (2007) Large-scale production of dsRNA and siRNA pools for RNA interference utilizing bacteriophage phi 6 RNAdependent RNA polymerase. RNA 13:422–429

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism and function. Cell 116:281–297.

Baulcombe D (2004) RNA silencing in plants. Nature 431:356–363.

Baumberger N, Baulcombe DC. Arabidopsis ARGONAUTE1 is an RNA Slicer that selectively recruits microRNAs and short interfering RNAs. Proc Natl Acad Sci U S A 2005;102:11928–33.

Behlke, M. (2008). Chemical modification of siRNAs for in vivo use. Oligonucleotides 18, 305–308.

Bennett, M., Deikman, J., Hendrix, B., and Iandolino, A. (2020). Barriers to Efficient Foliar Uptake of dsRNA and Molecular Barriers to dsRNA Activity in Plant Cells. Frontiers in Plant Science, 11(June), 1–7. https://doi.org/10.3389/fpls.2020.00816

Bollman KM, Aukerman MJ, Park MY, Hunter C, Berardini TZ, Poethig RS. HASTY, the Arabidopsis ortholog of exportin 5/ MSN5, regulates phase change and morphogenesis. Development 2003;130:1493–504.

Borah, M., Berbati, M., Reppa, C., Holeva, M., Nath, P. D., &Voloudakis, A. (2018). RNA-based vaccination of Bhut Jolokia pepper (Capsicum chinense Jacq.) against cucumber mosaic virus. Virusdisease, 29(2), 207-211.)

Caenorhabditis elegans. Gene 263:103–112 Cell Rep 29:1261–1268.

Chen, H.M., Chen, L.T., Patel, K., Li, Y.H., Baulcombe, D.C. and Wu, S.H. (2010) 22-nucleotide RNAs trigger secondary siRNA biogenesis in plants. Proc. Natl Acad. Sci. USA, 107, 15269–15274.

Christou, P. Rice transformation: bombardment. Plant Mol. Biol. 35, 197–203 (1997).

Ding XS et al (2006) Characterization of a brome mosaic virus strain and its use as a vector for gene silencing in monocotyledonous hosts. Mol Plant Microbe Interact 19(11): 1229–1239

Dubrovina, A. S., and Kiselev, K. V. (2019). Exogenous RNAs for gene regulation and plant resistance. International Journal of Molecular Sciences, 20(9). https://doi.org/10.3390/ijms20092282

Dunoyer P, Himber C, Voinnet O (2006) Induction, suppression and requirement of RNA silencing pathways in virulent Agrobacterium tumefaciens infections. Nat. Genet. 38(2):258-263.

Dutta, T. K., Banakar, P., and Rao, U. (2015). The status of RNAi-based transgenic research in plant nematology. Frontiers in Microbiology, 5, 760.

Eschen-Lippold, L., Landgraf, R., Smolka, U., Schulze, S., Heilmann, M., Heilmann, I., Hause, G., and Rosahl, S. (2012). Activation of defense against Phytophthora infestans in potato by down-regulation of syntaxin gene expression. New Phytologist, 193(4), 985–996. https://doi.org/10.1111/j.1469-8137.2011.04024.x

Eschen-Lippold, L., Landgraf, R., Smolka, U., Schulze, S., Heilmann, M., Heilmann, I., Hause, G., and Rosahl, S. (2012). Activation of defense against Phytophthora infestans in potato by down-regulation of syntaxin gene expression. New Phytologist, 193(4), 985–996. https://doi.org/10.1111/j.1469-8137.2011.04024.x

Fahlgren, N.; Montgomery, T.A.; Howell, M.D.; Allen, E.; Dvorak, S.K.; Alexander, A.L.; Carrington, J.C. Regulation of AUXIN RESPONSE FACTOR3 by TAS3 ta-siRNA affects developmental timing and patterning in Arabidopsis. Curr. Biol., 2006, 16(9), 939- 944.

Fei, Q.; Xia, R.; Meyers, B.C. Phased, secondary, small interfering RNAs in

posttranscriptional regulatory networks. Plant Cell, 2013, 25(7), 2400-2415.

Fire, A., S. Xu, M.K. Montgomery, S.A. Kostas, S.E. Driver, and C.C. Mello. 1998. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391: 806–811.

Gan D, Zhang J, Jiang H, Jiang T, Zhu S, Cheng B (2010) Bacterially expressed dsRNA protects maize against SCMV infection. Plant

Goodin MM, Zaitlin D, Naidu RA, Lommel SA (2008) Nicotiana benthamiana: Its history and future as a model for plant-pathogen interactions. Mol PlantMicrobe Interact 21: 1015–1026

Holzberg S et al (2002) Barley stripe mosaic virus-induced gene silencing in a monocot plant. Plant J 30(3):315–327

Huang, S., Iandolino, A., and Peel, G. (2018). Methods and Compositions for Introducing Nucleic Acids into Plants. U.S. Patent No. 20180163219A1. Washington, DC: U.S. Patent and Trademark Office.

Jacobs, T. B., Lawler, N. J., Lafayette, P. R., Vodkin, L. O., and Parrott, W. A. (2016). Simple gene silencing using the trans-acting siRNA pathway. Plant Biotechnology Journal, 14(1), 117–127. https://doi.org/10.1111/pbi.12362

Jaubert M, Bhattacharjee S, Mello AF, Perry KL, Moffett P. 2011. ARGONAUTE2 mediates RNA silencing antiviral defenses against Potato virus X in Arabidopsis. Plant Physiol. 156:1556–64

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

Kaldis, A., Berbati, M., Melita, O., Reppa, C., Holeva, M., Otten, P., &Voloudakis, A. (2018). Exogenously applied dsRNA molecules deriving from the Zucchini yellow mosaic virus (ZYMV) genome move systemically and protect cucurbits against ZYMV. Molecular plant pathology, 19(4), 883-895.

Kertbundit, S.; Pongtanom, N.; Ruanjan, P.; Chantasingh, D.; Tanwanchai, A.; Panyim, S.; Juricek, M. Resistance of transgenic papaya plants to papaya ringspot virus. Biol. Plant., 2007, 51, 333-339.

Kjemtrup S, Sampson KS, Peele CG, Nguyen LV, Conkling MA (1998). Gene silencing from plant DNA carried by a geminivirus. Plant J. 14:91-100.

Klahre, U., Crete, P., Leuenberger, S. A., Iglesias, V. A. and Meins, F. High molecular weight RNAs and small interfering RNAs induce systemic posttranscriptional gene silencing in plants. Proc. Natl Acad. Sci. USA 99, 11981–11986 (2002).

Konakalla, N. C., Kaldis, A., Berbati, M., Masarapu, H., &Voloudakis, A. E. (2016). Exogenous application of double-stranded RNA molecules from TMV p126 and CP genes confers resistance against TMV in tobacco. Planta, 244(4), 961-969.

Konakalla, N. C., Kaldis, A., Masarapu, H., &Voloudakis, A. E. (2019). Topical application of double stranded RNA molecules deriving from Sesbania mosaic virus (SeMV) CP and MP genes protects Sesbania plants against SeMV. European Journal of Plant Pathology, 155(4), 1345-1352.

Kozomara A, Griffiths-Jones S. MiRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res 2014; 42: D68–73.

Kurihara Y, Takashi Y, Watanabe Y (2006) The interaction between DCL1 and HYL1 is important for efficient and precise processing of pri-miRNA in plant microRNA biogenesis. RNA 12:206–212

Lange, M., Yellina, A. L., Orashakova, S., Becker, A., and Gene, V. (2013). Chapter 1 Virus-Induced Gene Silencing (VIGS) in Plants : An Overview of Target Species and the Virus-Derived Vector Systems. May 2016, 0–14. https://doi.org/10.1007/978-1-62703-278-0

Li S, Liu L, Zhuang X, Yu Y, Liu X, Cui X, et al. MicroRNAs inhibit the translation of target mRNAs on the endoplasmic reticulum in Arabidopsis. Cell 2013;153:562–74.

Li, F., Pignatta, D., Bendix, C., Brunkard, J.O., Cohn, M.M., Tung, J., Sun, H.Y., Kumar, P. and Baker, B. (2012) MicroRNA regulation of plant innate immune receptors. Proc. Natl Acad. Sci. USA 109,

1790–1795.

Lingel A, Simon B, Izaurralde E, Sattler M. 2004. Nucleic acid 3_-end recognition by the Argonaute2 PAZ domain. Nat. Struct. Mol. Biol. 11:576–77

Moldovan D, Spriggs A, Yang J, Pogson BJ, Dennis ES, Wilson IW. Hypoxia-responsive microRNAs and trans-acting small interfering RNAs in Arabidopsis. J Exp Bot 2010;61:165–77.

Moritoh S, Miki D, Akiyama M, Kawahara M, Izawa T, Maki H, Shimamoto K (2005) RNAi-mediated silencing of OsGEN-L (OsGEN-like), a new member of the RAD2/XPG nuclease family, causes male sterility by defect of microspore development in rice. Plant Cell Physiol. 46:699-715

Nicaise, V. and Candresse, T. (2017) Plum pox virus capsid protein suppresses plant pathogen-associated molecular pattern (PAMP)-triggered immunity. Mol. Plant Pathol. 18, 878–886.

Niehl, A., Soininen, M., Poranen, M. M., and Heinlein, M. (2018). Synthetic biology approach for plant protection using dsRNA. Plant Biotechnology Journal, 16(9), 1679–1687.

Orzaez D, Mirabel S, Wieland WH, Granell A (2006) Agroinjection of tomato fruits. A tool for rapid functional analysis of transgenes directly in fruit. Plant Physiol 140: 3–11.

Parent JS, Martı´nez de Alba AE, Vaucheret H. The origin and effect of small RNA signaling in plants. Front Plant Sci 2012;3:179.

Parker JS. 2010. How to slice: snapshots of Argonaute in action. Silence 1:3

Parry DH, Xu J, Ruvkun G (2007) A whole-genome RNAi screen for C. elegans miRNA pathway genes. Curr Biol 17:2013–2022.

Pérez-Amador, M. A., Abler, M. L., De Rocher, E. J., Thompson, D. M., van Hoof, A., LeBrasseur, N. D., et al. (2000). Identification of BFN1, a bifunctional nuclease induced during leaf and stem senescence in Arabidopsis. Plant Physiol. 122, 169–179.

Purkayastha A et al (2010) Virus-induced gene silencing in rice using a vector derived from a DNA virus. Planta 232(6):1531–1540

Purkayastha A, Dasgupta I (2009) Virus induced gene silencing: a versatile tool for discovery of gene functions in plants. Plant Physiol Biochem 47(11–12):967–976

Ramachandran V and Chen X (2008). Degradation of microRNAs by a family of exoribonucleases in Arabidopsis. Science 2008; 321: 1490–2.

Robinson, K. E., Worrall, E. A., and Mitter, N. (2014). Double stranded RNA expression and its topical application for non-transgenic resistance to plant viruses. Journal of Plant Biochemistry and Biotechnology, 23(3), 231–237. https://doi.org/10.1007/s13562-014-0260-z

Rogers, K., and Chen, X. (2013). Biogenesis, turnover, and mode of action of plant microRNAs. Plant Cell, 25(7), 2383–2399. https://doi.org/10.1105/tpc.113.113159

Rosa, C., Kuo, Y.-W., Wuriyanghan, H., and Falk, B. W. (2018). RNA Interference Mechanisms and Applications in Plant Pathology. Annual Review of Phytopathology, 56(1), 581–610. https://doi.org/10.1146/annurev-phyto-080417-050044

S. Pflieger, S. Blanchet, L. Camborde, et al., “Efficient virus induced gene silencing in Arabidopsis using a ‘one-step’ TYMV-derived vector,” The Plant Journal, vol. 56, no. 4, pp. 678–690, 2008.

Santos-Rosa M, Poutaraud A, Merdinoglu D, Mestre P (2008) Development of a transient expression system in grapevine via agro-infiltration. Plant Cell Reports 27: 1053–1063

Schreiber, L. (2005). Polar paths of diffusion across plant cuticles new evidence for an old hypothesis. Ann. Bot. 95, 1069–1073. doi: 10.1093/aob/ mci122.

Sco fi eld SR et al (2005) Development of a virus-induced gene-silencing system for hexaploid wheat and its use in functional analysis of the Lr21-mediated leaf rust resistance pathway. Plant Physiol 138(4):2165–2173

Shivaprasad, P.V., Chen, H.M., Patel, K., Bond, D.M., Santos, B. and Baulcombe, D.C.

(2012) A microRNA superfamily regulates nucleotide binding site-leucine-rich repeats and other mRNAs. Plant Cell, 24, 859–874.

Song, X.W., Li, P.C., Zhai, J.X., Zhou, M., Ma, L.J., Liu, B., Jeong, D.H., Nakano, M., Cao, S.Y., Liu, C.Y., Chu, C.C., Wang, X.J., Green, P.J., Meyers, B.C. and Cao, X.F. (2012) Roles of DCL4 and DCL3b in rice phased small RNA biogenesis. Plant J. 69, 462–474.

Tenllado, F., and Díaz-Ruíz, J. R. (2001). Double-Stranded RNA-Mediated Interference with Plant Virus Infection. Journal of Virology, 75(24), 12288–12297.

Timmons L, Court DL, Fire A (2001) Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans.* Gene 263:103–112.

Voinnet O, Pinto YM, Baulcombe DC. Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. Proc Natl Acad Sci USA 1999;96:14147–52.

Waterhouse PM and Helliwell CA (2003) Exploring plant genomes by RNAinduced gene silencing. Nat Rev Genet 4: 29–38.

Watson, J.M, Fusaro, A.F, Wang. M.; Waterhouse, P.M. RNA silencing platforms in plants. FEBS Lett., 2005, 579, 5982-5987.

Whitham, S., Dinesh-Kumar, S. P., Choi, D., Hehl, R., Corr, C., and Baker, B. 1994. The product of the tobacco mosaic virus resistance gene N: similarity to toll and theinterleukin-1 receptor. Cell 78:1101-1115.

Wroblewski T, Tomczak A, Michelmore R (2005) Optimization of Agrobacterium mediated transient assays of gene expression in lettuce, tomato and Arabidopsis. Plant Biotech J 3: 259–273.

Xie, Z.; Allen, E.; Wilken, A.; Carrington, J.C. DICER-LIKE 4 functions in trans-actingsmall interfering RNA biogenesis and vegetative phase change in Arabidopsis thaliana. Proc. Natl.

Y. Liu, M. Schiff, and S. P. Dinesh-Kumar, "Virus-induced gene silencing in tomato," The Plant Journal, vol. 31, no. 6, pp. 777–

Yang L, Liu Z, Lu F, Dong A, Huang H (2006a) SERRATE is a novel nuclear regulator in primary microRNA processing

Yang YN, Li RG, Qi M (2000) In vivo analysis of plant promoters and transcription factors by agroinfiltration of tobacco leaves. Plant J 22: 543–551

Yin Y, Chory J, Baulcombe D. RNAi in transgenic plants. Curr Protoc Mol Biol. 2005; Chapter 26:. doi:10.1002/0471142727.mb2606s72.

Zhang H, Xia R, Meyers BC, Walbot V. 2015. Evolution, functions, and mysteries of plant ARGONAUTE proteins. Curr. Opin. Plant Biol. 27:84–90

Zhu H, Zhou Y, Castillo-González C, Lu A, Ge C et al (2013) Bidirectional processing of pri-miRNAs with branched terminal loops by Arabidopsis Dicer-like1. Nat Struct Mol Biol 20:1106–1115.

How to cite this article:

Gowthamkumar Routhu, Munmi Borah, P. D. Nath and Bisakha Deb. 2020. RNA Interference (RNAi) and Response of Plant Cells to Double Stranded RNA (dsRNA). *Int.J.Curr.Microbiol.App.Sci.* 9(09): 3114-3125. doi: https://doi.org/10.20546/ijcmas.2020.909.384