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The function of small RNAs in plant biotic stress response

Juan Huang†, Meiling Yang† and Xiaoming Zhang*

State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China.
†These authors contributed equally to this work.

Invited Expert Review

Xiaoming Zhang
*Correspondence:
zhangxm@ioz.ac.cn

Abstract Small RNAs (sRNAs) play essential roles in plants upon biotic stress. Plants utilize RNA silencing machinery to facilitate pathogen-associated molecular pattern-triggered immunity and effector-triggered immunity to defend against pathogen attack or to facilitate defense against insect herbivores. Pathogens, on the other hand, are also able to generate effectors and sRNAs to counter the host immune response. The arms race between plants and pathogens/insect herbivores has triggered the evolution of sRNAs, RNA silencing machinery and pathogen effectors. A great number of studies have been performed to investigate the roles of sRNAs in plant defense, bringing in the opportunity to utilize sRNAs in plant protection. Transgenic plants with pathogen-derived resistance ability or trans-generational defense have been generated, which show promising potential as solutions for pathogen/insect herbivore problems in the field. Here we summarize the recent progress on the function of sRNAs in response to biotic stress, mainly in plant-pathogen/insect herbivore interaction, and the application of sRNAs in disease and insect herbivore control.

Keywords: Small RNA; plant immunity; transgenerational defense; pathogen-derived resistance
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INTRODUCTION

With the ever-increasing world population and the loss of agricultural land, it is crucial to find means to improve global food production. Biotic threats to food growth and transport include bacteria, fungi, oomycetes, viruses and insect pests. All these together account for up to 30% loss of the world's crops both pre- and post-harvest (Oerke 2006; Flood 2010; Bebber and Gurr 2015). Therefore, it is important to uncover the biotic stress responses in plants and develop novel tools to protect crops from pathogens and pests. Plant pathogens all challenge the immune system of the plant. To counter pathogen infection, plants have evolved a defense response by activating or suppressing a large array of genes (Jones and Dangl 2006). Upon pathogen attack, an array of pathogen-associated molecular patterns (PAMPs) or host danger-associated molecular patterns (DAMPs) are recognized by plants (Zvereva and Pooggin 2012). Plants use cell-surface localized pattern-recognition receptors (PRRs) to detect PAMP or DAMP triggered by pathogens. For instance, flagellin-sensing 2 (FLS2) and elongation factor-TU (EF-Tu) receptor (EFR) detect bacterial flagellin and EF-Tu, respectively, while chitin-elicitor receptor kinase 1 (CERK1) and lysin motif receptor kinase 5 (LYK5) both detect fungi chitin (Gomez-Gomez and Boller 2000; Zipfel et al. 2006; Shimizu et al. 2010; Cao et al. 2014). PAMP or DAMP activates PAMP-triggered immunity (PTI), which involves the induction of callose deposition, production of reactive oxygen species, accumulation of salicylic acid (SA), and expression of pathogenesis-related (PR) genes (Yang and Huang 2014). However, successful pathogens have evolved protein effectors to suppress PTI, resulting in effector-triggered susceptibility (ETS) (Dou and Zhou 2012; Feng and Zhou 2012). In turn, plants have developed a secondary immune response, known as effector-triggered immunity (ETI). ETI is triggered by resistance (R) proteins that can recognize specific pathogen effectors and suppress them. R proteins usually trigger a more robust and specific response such as hypersensitive response (HR), which mediates cell death at the sites of infection to limit the growth of the pathogen. Pathogens have diversified their effectors to induce another round of ETS, while plants evolved new R proteins to recognize the new effectors. This war of defense and counter-defense between host and pathogen has resulted in the diverse array of pathogen effectors and resistance genes (Tsuda and Katagiri 2010; Liu et al. 2014b; Bigeard et al. 2015).

Small RNAs (sRNAs) are 20 to 30 nucleotide (nt)-long noncoding RNA molecules that regulate gene expression in eukaryotes through a process generally termed RNA silencing (Zamore and Haley 2005; Chapman and Carrington 2007). They

are distinguished by their precursor structure and biogenesis pathway, and in plants, sRNAs are divided into two major classes: microRNA (miRNA) and small interfering RNA (siRNA). miRNAs are usually 21–24 nt long and are derived from RNAs with imperfectly base-paired hairpin structures (Chen 2009). siRNAs are generated from perfectly complementary long double-stranded RNAs (dsRNAs) and may require RNA-dependent RNA polymerases (RDRs) (Bartel 2009; Katiyar-Agarwal and Jin 2010). There are several siRNA subclasses present in plants, including *trans*-acting siRNAs (ta-siRNAs), heterochromatic siRNAs (hc-siRNAs), natural antisense transcript-derived siRNAs (nat-siRNAs), and long siRNAs (lsiRNAs). sRNAs induce gene regulation in hosts or pathogens by post-transcriptional gene silencing (PTGS) or transcriptional gene silencing (TGS). Both miRNAs and siRNAs can induce PTGS by messenger RNA (mRNA) cleavage/degradation or translational inhibition via a RNA-induced silencing complex (RISC), while TGS, which results in either DNA methylation, histone modification or chromatin modification, is usually mediated by siRNAs and some specific miRNAs (Baulcombe 2004; Chellappan et al. 2004; Vaucheret 2006; Wu et al. 2010; Cui and Cao 2014). The biogenesis pathways of different sRNAs can be complicated and species-specific, and while they have some steps in common, many steps are unique to certain sRNAs. For detailed biogenesis information on various sRNAs, several reviews are available (Ding and Voinnet 2007; Ding 2010; Katiyar-Agarwal and Jin 2010; Rogers and Chen 2013; Weiberg et al. 2014).

Numerous studies have shown that RNA silencing machinery plays critical roles in PTI and ETI. In this review, we will summarize the recent progress on the function of sRNAs in response to biotic stress to mediate defense, particularly during plant-pathogen/insect herbivore interactions. We will also discuss the application of sRNAs in disease and insect herbivore control.

sRNAS IN PLANT-PATHOGEN/INSECT HERBIVORE INTERACTION

Plants have developed complicated defense systems in response to various pathogen attacks, while pathogens have also evolved diverse effectors or suppressors as counter defenses. The result of plant-pathogen interactions depends on the relative contribution of susceptibility and resistance factors. In this section, we discuss the roles of sRNAs in both plant immunity and pathogen infection. The targets and function of sRNAs in response to different pathogen stressors are summarized (Table 1). In addition, recent discovery shows that plants produce sRNAs in response to insect herbivore attack. Thus, we also discuss the function of sRNA in plant-insect herbivore interaction.

sRNAs play a role in host PTI against bacteria, fungi and oomycetes

After penetration through the plant cell wall, bacteria, fungi and oomycetes localize in the intercellular space for amplification. Fungi and oomycetes also enter into the cells in the later infection stages. Entry of these microbes immediately activates the host PTI response. PTI requires miRNAs and siRNAs, which act as key fine-tuning regulators of plant hormones, including auxin, abscisic acid (ABA), SA and jasmonic acid (JA) (Figure 1A) (Zhang et al. 2011a).

The first miRNA identified to be involved in PTI is *Arabidopsis* miR393 (Navarro et al. 2006). In response to bacteria pathogen *Pseudomonas syringae* attack, miR393 was induced by a flagellin-derived peptide, flg22. miR393 then suppresses auxin signaling by negatively regulating mRNAs of auxin receptors, transport inhibitor response 1 (TIR1), AFB2 and AFB3, which allows plants to prioritize defense signaling over plant growth, and triggers a series of defense responses (Navarro et al. 2006). SA is responsible for defense against biotrophic pathogens, while glucosinolates are anti-microbial molecules that contribute to plant defense against pests and diseases. Further studies revealed that the action of miR393 on auxin signaling can prevent the suppression of SA, increase glucosinolate levels, and decrease camalexin levels, which subsequently enhances the resistance of *Arabidopsis* to *P. syringae* (Robert-Seilaniantz et al. 2011). Furthermore, miR393 functions the same way as auxin signaling in other plants, such as rice (*Oryza sativa*) (Bian et al. 2012; Xia et al. 2012). In rice, miR393 targets both *OsTIR1* and *OsAFB2*, and over-expressing miR393 results in increased tillers, early flowering, reduced tolerance to salt and drought and hyposensitivity to auxin (Bian et al. 2012; Xia et al. 2012). In addition to miR393, the expression of other miRNAs can be differentially activated or repressed by flg22 to further regulate disease resistance (Jagadeeswaran et al. 2009; Li et al. 2010; Zhang et al. 2011a). miRNAs such as miR158, miR160, miR167, miR156, miR398 and miR773, exhibited over 30% increase or decrease in expression after flg22 treatment. miR160a was up-regulated during flg22 treatment, whereas miR398b and miR773 were down-regulated (Li et al. 2010). miR167 was induced in *Pst* DC3000-, *Pst avrRpt2*- and *Pst hrcC*-challenged plants, while miR390a was down-regulated in response to a virulent strain of *Pst* DC3000 (Zhang et al. 2011a). These bacterial-regulated miRNAs play important roles in plant defense by targeting genes involved in plant hormone biosynthesis and signaling pathways. miR160a targets auxin response factors ARF16 and ARF17 and induces callose deposition (Li et al. 2010). In the orange tree (*Citrus sinensis* (L.) Osbeck), miR399 was specifically induced by the infection of *Candidatus* L. asiaticus, a bacterium that causes Huanglongbing (HLB), also known as citrus greening disease. Further experiments suggest that the increase of miR399 may be a result of phosphorus deficiency caused by HLB (Zhao et al. 2013). miR408 in wheat and *Arabidopsis* is shown to target genes encoding plantacyanin-like proteins. miR408 in wheat (*Triticum aestivum* L.) negatively regulates the expression of *TaCLP1*, a type of plantacyanin, and increases the vulnerability of wheat to stripe rust (Feng et al. 2013).

The role of miRNAs in PTI has also been demonstrated in fungal and oomycetal infection. osa-miR7695 was found to accumulate in rice treated with blast fungal mycelia (Campo et al. 2013). miR169a, miR172a and miR398b were involved in the basal response of rice challenged with fungus *M. oryzae* (Li et al. 2014b). The powdery mildew fungus *Blumeria graminis* triggered the generation of many miRNAs in wheat *T. aestivum*, among which miR167, miR171, miR444, miR408 and miR1138 are probably involved in PTI (Gupta et al. 2012). miR403 was down-regulated by the infection with oomycete *P. sojae* in soybean. The down-regulation of miR403 was

Table 1. sRNAs known to be involved in plant-pathogen interactions

Small RNA	Small RNA source	Host/pathogen	Target genes	Expression of gene upon infection	Roles in plant-pathogen interaction	References
miR159	Plant	*Arabidopsis*/Bacteria *P. syringae*	MYB33, MYB65, and MYC101	Up	Regulate gibberellin (GA) and ABA signaling pathways.	Zhang et al. 2011a
miR160	Plant	*Arabidopsis*/Bacteria *P. syringae*	ARF10, ARF16, and ARF17	Up	Increase PAMP-induced callose deposition.	Li et al. 2010
	Plant	*M. esculenta*/Fungus *C. gloeosporioides*	ARF10	Up	Regulate plant auxin and enhance plant defense responses.	Pinweha et al. 2015
	Plant	*O. sativa*/Fungus *M. oryzae*	ARF16 and a B3 DNA-binding domain-containing protein	Up	Over-expression of miR160 increases the accumulation of hydrogen peroxide and defense-related genes and attenuates fungal growth.	Li et al. 2014b
miR167	Plant	*Arabidopsis*/Bacteria *P. syringae*	ARF8, ARF6	Up	Regulate auxin signaling pathway and enhance plant defense response.	Fahlgren et al. 2007; Zhang et al. 2011a
miR168	Plant	*O. sativa*/Viruses RSV and RDV	AGO1	?	Infection induces accumulation of AGO18 which sequesters miR168. AGO1 expression is then rescued, resulting in enhanced plant defense.	Wu et al. 2015
miR390	Plant	*Arabidopsis*/Bacteria *P. syringae*	TAS3	Down	Trigger the accumulation of ta-siRNAs that regulate the expression of *ARF3* and *ARF4*, genes involved in auxin signaling.	Zhang et al. 2011a
miR393	Plant	*Arabidopsis*/Bacteria *P. syringae*	TIR1, AFB2, and AFB3	Up	Regulate auxin signaling and enhance plant defense response.	Navarro et al. 2006; Fahlgren et al. 2007
	Plant	*M. esculenta*/Fungus *Colletotrichum gloeosporioides*	TIR1	Up	Regulate auxin signaling and enhance plant defense response.	Pinweha et al. 2015
miR393b*	Plant	*Arabidopsis and Nicotiana benthamiana*/ Bacteria *P. syringae*	MEMB12	Up	Increase the secretion of antimicrobial pathogenesis-related protein PR1.	Zhang et al. 2011b
miR396a-5p	Plant	*Solanaceae*/Oomycete *P. infestans*	GRF	Down	Over-expression of miR396a-5p decreases plant resistance to *P. nicotianae*.	Chen et al. 2015
miR398	Plant	*Arabidopsis*/Bacteria *P. syringae*	COX5b.1, CSD1 and CSD2	Down	Negatively regulate callose deposition and is involved in the suppression of auxin signaling and detoxification of ROS.	Jagadeeswaran et al. 2009; Li et al. 2010
	Plant	*Hordeum vulgare L.*/ Fungus *Blumeria graminis* f. sp. *hordei*	SOD1	?	Mla and Rom repress miR398-mediated SOD1 expression to change the HR response to fungus.	Kerchev et al. 2013
	Plant	*O. sativa*/Fungus *M. oryzae*	SOD2	Up	Over-expression of miR398 increases the accumulation of hydrogen peroxide and defense-related genes and decreases fungal growth.	Li et al. 2014b

(Continued)

Table 1. (Continued)

Small RNA	Small RNA source	Host/pathogen	Target genes	Expression of gene upon infection	Roles in plant-pathogen interaction	References
miR399	Plant	Citrus/Bacteria *Ca.* L. asiaticus	PHO2	Up	Contribute to HLB symptoms and phosphorus homeostasis and signaling.	Zhao et al. 2013
miR408	Plant	*Arabidopsis*/Bacteria *P. syringae*	Copper protein plantacyanin, laccase copper protein and copper ion binding protein genes (predicted targets)	Up/Down	?	Zhang et al. 2011a
	Plant	*Wheat*/Fungus *Puccinia striiformis* f. sp. tritici	TaCLP1, a type of plantacyanin protein	Up/Down	Negatively regulate wheat resistance to stripe rust.	Feng et al. 2013
miR472	Plant	*Arabidopsis*/Bacteria *P. syringae*	CC-NBS-LRR	?	Over-expression of miR472 decreases plant resistance to bacteria.	Boccara et al. 2014
miR482	Plant	*S. lycopersicum*/Viruses TCV, CMV and TRV	NBS-LRR	Down	Virus and bacteria infection down-regulates the expression of miR482 and induces the expression of R protein.	Shivaprasad et al. 2012
	Plant	*G. raimondii*/Fungus *V. dahliae*	NBS-LRR	Down	Fungal pathogen infection down-regulates the expression of miR482 and induces the expression of R protein.	Zhu et al. 2013
	Plant	*S. lycopersicum*/Fungus *F. oxysporum*	*Solyc08g075630*, *Solyc08g076000*	Down	Fungus infection down-regulates the accumulation of miR482 to increase the expression of NB domain genes.	Ouyang et al. 2014
miR773	Plant	*Arabidopsis*/Bacteria *P. syringae*	*MET2*	Down	Negatively regulate callose deposition and disease resistance to bacteria.	Li et al. 2010
miR825	Plant	*Arabidopsis*/Bacteria *P. syringae*	Remorin, zinc finger homeobox family, frataxin-related	Up	?	Fahlgren et al. 2007
miR1507	Plant	*M. truncatula*/?	NBS-LRR	?	?	Zhai et al. 2011
miR1885	Plant	*Brassica napus*/Virus TuMV	TIR–NBS–LRR	Up	Repress ETI	Wroblewski et al. 2007
miR2109	Plant	*Medicago*/?	NBS-LRR	?	?	Zhai et al. 2011
miR2118	Plant	*Medicago*/?	NBS-LRR	?	?	Zhai et al. 2011
	Plant	*S. lycopersicum*/Viruses TCV, CMV and TRV	NBS-LRR	Down	Virus and bacteria infection down-regulates the expression of miR482 and induces the expression of R protein.	Shivaprasad et al. 2012

(Continued)

Table 1. (Continued)

Small RNA	Small RNA source	Host/pathogen	Target genes	Expression of gene upon infection	Roles in plant-pathogen interaction	References
miR5300	Plant	*S. lycopersicum*/Fungus *F. oxysporum*	*Solyc05g008650*, *tm-2*	Down	Fungus infection down-regulates the accumulation of miR5300 to increase the expression of NB domain genes.	Ouyang et al. 2014
miR6019/miR6020	Plant	*N. tabacum*/Virus TMV	TIR-NBS-LRR	?	Over-expression of miR6019 and miR6020 attenuates N-gene mediated resistance to viruses.	Li et al. 2012
miR7695	Plant	*O. sativa*/Fungus *M. oryzae*	OsNramp6	?	Over-expression of miR7695 enhances plant defense resistance.	Campo et al. 2013
miR9863	Plant	*Hordeum vulgare* L./ Fungus *Blumeria graminis* f. sp. *hordei*	Mla1	?	Over-expression of miR9863 reduces fungal resistance and cell-death signaling.	Liu et al. 2014a
nat-siRNAATGB2	Plant	*Arabidopsis*/Bacteria *P. syringae*	PPRL	Up	Contribute to plant immunity by suppressing a negative regulator of the RPS2 pathway.	Katiyar-Agarwal et al. 2006
AtlsiRNA-1	Plant	*Arabidopsis*/Bacteria *P. syringae*	AtRAP	Up	Contribute to plant immunity by silencing a negative regulator.	Katiyar-Agarwal et al. 2007
Bc-siR3.1	Pathogen	*Arabidopsis* and *S. lycopersicum*/Fungus *Botrytis cinerea*	PRXIIF	?	Silence host immunity genes.	Weiberg et al. 2013
Bc-siR3.2	Pathogen	*Arabidopsis* and *S. lycopersicum*/Fungus *B. cinerea*	MPK2 and MPK1	?	Silence host immunity genes.	Weiberg et al. 2013
Bc-siR5	Pathogen	*Arabidopsis* and *S. lycopersicum*/Fungus *B. cinerea*	WAK	?	Silence host immunity genes.	Weiberg et al. 2013
TMV vsiRNA	Pathogen	*Arabidopsis*/Virus TMV	CPSF30, TRAPa	?	?	Qi et al. 2009
Y-Sat siRNA	Pathogen	*N. tabacum*/Y satellite (Y-sat) RNA of CMV	CHLI	?	Target host chli genes to induce yellowing symptoms.	Shimura et al. 2011; Smith et al. 2011
PC-sRNA8a/PC-sRNA8b	Pathogen	*P. persica*/Viroid *PLMVd*	HSP90	?	Target HSP90 and contribute to chloroplast biogenesis and signal transduction.	Navarro et al. 2012
vd39/vd40	Pathogen	*S. lycopersicum*/Viroid *PSTVd*	CalS11-like and CalS12-like	?	Target CalS11-like and CalS12-like to induce infection phenotypes.	Adkar-Purushothama et al. 2015
vdsiRNA	Pathogen	*S. lycopersicum*/Viroid *TPMVd*	SolWD40	?	?	Avina-Padilla et al. 2015

ABA, abscisic acid; AGO, argonaute; ARF, auxin response factors; CC-NBS-LRR, coiled-coil nucleotide-binding site-leucine-rich repeat; CDS, copper/zinc superoxide dismutase gene; CHLI, chelatase subunit I; CPSF, polyadenylation specificity factor; ETI, effector-triggered immunity; GRF, growth-regulating factor; HLB, Huanglongbing; HSP, heat shock protein; Mla, Mildew resistance locus a; MPK, mitogen activated protein kinase; NB, nucleotide-binding; Nramp6, natural resistance-associated macrophage protein 6; PPRL, pentatricopetide repeats-like; PR, pathogenesis-related; PRXIIF, peroxiredoxin; R, resistance; RAP, RNAbinding domain abundant in Apicomplexans; ROS, reactive oxygen species; SOD, superoxide dismutase; TAS, trans-acting siRNA; TIR, transport inhibitor response; TRAP, translocon-associated protein alpha.

correlated with the increase of the expression of its target genes (Guo et al. 2011).

Pathogen effectors and sRNAs facilitate infection by suppressing host PTI

In response to plant immunity, bacteria, fungi and oomycetes have all evolved effectors to interfere with host defense responses and enhance infection. Many effectors have been identified. For instance, *P. syringae* secretes more than 30 effectors (Lozano-Duran et al. 2014). The function and the mode of action of effectors are well-summarized in many reviews (Dou and Zhou 2012; Feng and Zhou 2012). Here, we focus on effectors that suppress host PTI by interfering with RNA silencing machinery. We also discuss some pathogen sRNAs that are delivered into the host cells as effectors to interfere with host PTI.

Pathogen effectors interfere with RNA silencing machinery by regulating the accumulation of sRNAs (Navarro et al. 2008). AvrPtoB, an effector with E3-ubiquitin ligase activity, suppresses miR393a and miR393b transcription by inhibiting the accumulation of pri-miR393a and pri-miR393b. AvrPto, which inhibits the kinase activity of multiple transmembrane PRRs to suppress PTI, can also interfere with PTI by reducing miR393 accumulation at the post-transcriptional level (Navarro et al. 2008). In the oomycete *P. sojae*, two effectors, Phytophthora suppressors of RNA silencing 1 and 2 (PSR1 and PSR2), were found to reduce the accumulation of sRNAs (Qiao et al. 2013; Qiao et al. 2015). PSR1 can decrease levels of miRNAs and endogenous siRNAs by binding to a conserved nuclear protein PSR1-Interacting Protein 1 (PINP1). PINP1 is required for the accumulation of distinct classes of sRNAs, most likely by facilitating the assembly of dicing complexes (Qiao et al. 2015). On the other hand, PSR2 is involved in decreasing the accumulation of specific ta-siRNAs, ASRP255 and ASRP1151 (Qiao et al. 2013). PSR2 can target miR173 to suppress the biosynthesis of ASRP255 and ASRP1151 ta-siRNAs, but not affect miR390-mediated *TAS3* ta-siRNAs (Qiao et al. 2013). In addition to suppressing sRNA accumulation, effectors can also interfere with the process of RNA silencing. For instance, HopT1-1 from *Pst* DC3000 was shown to interfere with miRNA-directed translational inhibition, most likely by suppressing AGO1-mediated silencing (Navarro et al. 2008). Furthermore, a recent study showed that sRNAs encoded by pathogens can be utilized to suppress host PTI. sRNAs, Bc-siR3 and Bc-siR5 which were transferred from *B. cinerea* to a host plant can hijack host AGO1 protein and subsequently suppress RNA silencing (Weiberg et al. 2013). In broad terms, both Bc-siR3 and Bc-siR5 can be considered as a special type of pathogen effector.

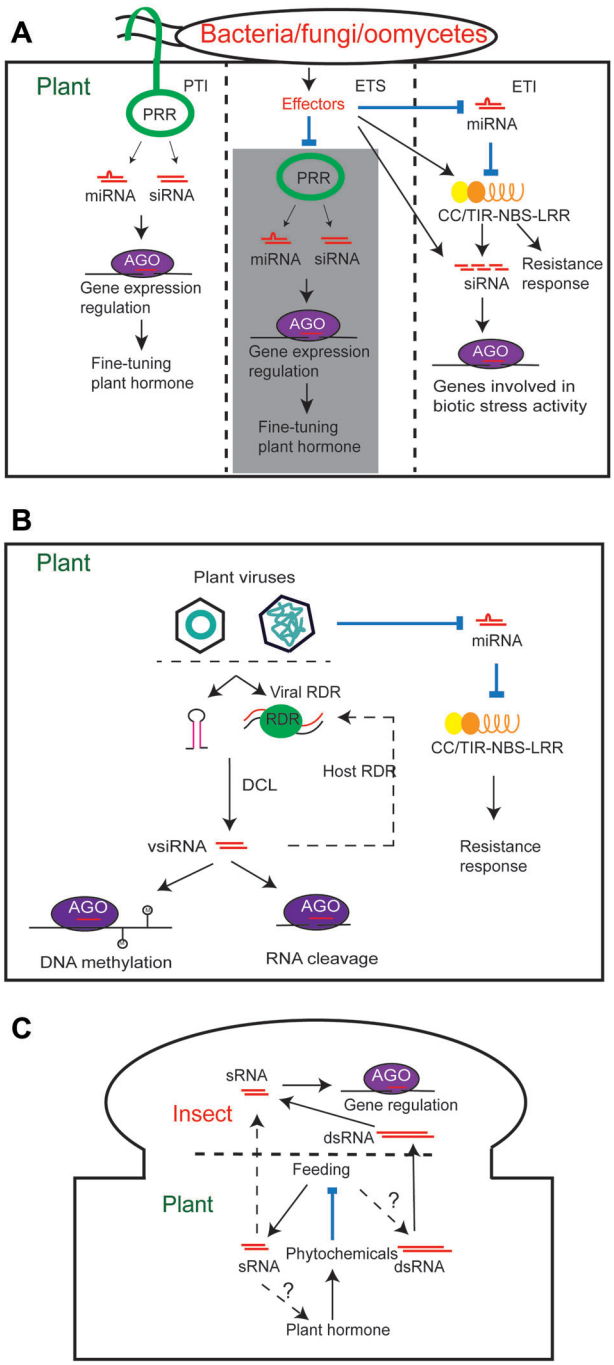

Figure 1. Continued.

Figure 1. The role of sRNAs in plant immunity

(A) The role of small RNAs (sRNAs) during interaction between plant and pathogens (bacteria/fungi/oomycetes). **(B)** Plants defend against virus attack by silencing viral DNA/RNA genome through RNAi. Both microRNAs (miRNAs) and virus-derived small interfering RNAs (vsiRNAs) are involved. **(C)** In response to insect herbivore attack, plants generate sRNAs that regulate the production of phytochemicals that are toxic to insect herbivores or increase plant resistance response. Whether plants produce natural sRNAs/dsRNAs (double-stranded RNAs) that target insect messenger RNAs remains an unanswered question. CC, coiled-coil; PTI, pathogen-associated molecular pattern-triggered immunity; ETS, effector-triggered susceptibility; ETI, effector-triggered immunity; PRR, pattern-recognition receptor; DCL, dicer-like protein; HEN 1, HUA ENHANCER 1; LRR, leucine-rich repeat; NBS, nucleotide-binding site; RDR, RNA-dependent RNA polymerase; TIR, transport inhibitor response; AGO, argonaute.

Host sRNAs facilitate plant ETI to defend pathogens

In order to overcome the problem of pathogen effectors, plants evolved *R* gene-mediated immunity, also termed ETI. R proteins can recognize pathogen effectors and trigger robust cellular changes, usually generating a HR at the infection site. Most plant *R* genes belong to the nucleotide-binding site (NBS)-leucine-rich repeat (LRR) gene family. Hundreds of diverse NBS-LRRs are encoded in plant genomes to allow the recognition of many pathogens (Meyers et al. 2005). Under normal conditions, the quantity and activity of R protein are maintained at a low level to save resources for plant growth and development. However, when plants are under attack, pathogen effectors can suppress PTI, leading to the up-regulation of R genes in plants, which subsequently trigger ETI.

sRNAs are involved in targeting and regulating the expression of *R* genes. In *Arabidopsis*, several *RPP5* (*recognition of peronosporaparasitica 5*) locus *R* genes, including *RPP4* and *SNC1* (suppressor of NPR1-1, constitutive 1) are up-regulated in *dcl4* and *ago1* mutants or in plants over-expressing viral suppressors of RNA silencing (VSRs) (Yi and Richards 2007). Moreover, to increase regulatory efficiency, miRNAs are designed to target the conserved region of NBS-LRRs, allowing one miRNA to target numerous *NBS-LRR* genes to suppress their expression (Zhai et al. 2011; Shivaprasad et al. 2012). In addition, some miRNAs can trigger the biogenesis of secondary siRNAs, which also regulate target gene expression and enhance the regulatory effect (Zhai et al. 2011; Manavella et al. 2012). In *Medicago truncatula*, three 22-nt miRNA families (miR1507, miR2109 and miR2118) are determined to target conserved domains in NBS-LRRs and trigger the production of phasiRNAs (Zhai et al. 2011). In tobacco (*N. benthamiana*), TIR-NBS-LRR (TNL)-type receptor genes are regulated by miR6019 and miR6020, while in tomato (*Solanum lycopersicum*), miR482/miR2118 are the predominant members that regulate *R* genes. In both cases, the cleavage caused by miRNAs triggers the biogenesis of phasiRNAs, which reinforces the suppression of *R* genes (Li et al. 2012; Shivaprasad et al. 2012). Although many miRNAs are down-regulated in resistant tomato cultivars upon fungal *Fusarium oxysporum* treatment, miR398, miR482 and miR5300 are induced. *tm-2* and another three NB domain genes, the targets of miR482 and miR5300, are induced in resistant but not susceptible tomato cultivars that were treated with *F. oxysporum*, indicates that miR482/miR5300-meidated *NB* gene regulation plays important roles in tomato resistance to fungi (Ouyang et al. 2014). miR472 in *Arabidopsis* is also shown to modulate both PTI and ETI through post-transcriptional control of coiled-coil NBS-LRR (CC-NBS-LRR) genes (Boccara et al. 2014). In addition, in barley (*Hordeum vulgare* L.), the miR9863 family and their triggered 21-nt phasiRNAs together form a regulation network to repress the expression of group 1 *Mla* alleles, which encode CC-NBS-LRR receptors. The over-expression of miR9863 reduced the MLA1-triggered powdery mildew fungus resistance and cell-death signaling (Liu et al. 2014a).

In addition to miRNAs and secondary siRNAs, other sRNAs also regulate ETI. *Arabidopsis* nat-siRNAATGB2, the first example of a plant endogenous siRNA, acts as a positive regulator in *avrRpt2*-triggered ETI (Katiyar-Agarwal et al. 2006). During infection, the avrRpt2 effector was recognized by R protein RPS2. Together with nonspecific disease resistance 1 (NDR1), RPS2 triggered the biogenesis of natsiRNAATGB2. natsiRNAATGB2 silences pentatricopeptide repeats-like (PPRL) and prevents the negative regulatory effects of PPRL on the RPS2 resistance pathway. Another siRNA induced by effector avrRpt2 is AtlsiRNA-1 (Katiyar-Agarwal et al. 2007). AtlsiRNA-1 is a 30–40-nt long siRNA (lsiRNA), which is generated from the SRRLK/AtRAP NAT pair. It silences *AtRAP* mRNA, most likely by decapping and XRN4-mediated 5′-to-3′ degradation. *AtRAP* encodes a RAP domain-containing protein involved in disease resistance. The silencing of the *AtRAP* results in the enhanced resistance to infection (Katiyar-Agarwal et al. 2007). It was once thought that the complementary strand of the miRNA, termed the miRNA* strand, was a useless by-product that was eventually degraded. However, a recent study showed that miR393b* is loaded into AGO2 to suppress the expression of *MEMB12* gene (Zhang et al. 2011b). *MEMB12* encodes a golgi-localized SNARE protein, which regulates the transportation of antimicrobial pathogenesis-related protein PR1. The silencing of *MEMB12* leads to increase of exocytosis of PR1, which subsequently enhances the antimicrobial activity of the plant. It has been uncovered that miR393, the pairing strand of miR393b*, is loaded into AGO1 and involved in the auxin immunity pathway (Navarro et al. 2008). Thus, two sRNAs generated from the same duplex facilitate plant resistance progress through different AGOs and pathways.

Direct anti-viral resistance of RNA silencing in plants cells

While bacteria replicate in the host intercellular space and fungi and oomycetes also localize there in an earlier infection stage, virus infections involve viral DNA or RNA replication and transcription inside the plant cell. PTI-based antiviral responses are most likely triggered by plant DAMPs (Zvereva and Pooggin 2012). Viral-encoded proteins, which were recognized by R protein and the RNA silencing system, could also elicit antiviral defenses against viruses (Soosaar et al. 2005; Moffett 2009). Anti-viral immunity triggers the production of virus-derived small interfering RNAs (vsiRNAs) to target and eliminate the RNA genome of the invading virus (Figure 1B). vsiRNAs was first detected in tobacco infected with Potato virus X (PVX) by Hamilton and Baulcombe (1999). 21–24-nt sRNAs complementary to the positive strand of PVX accumulated in infected leaves. Subsequent studies have demonstrated vsiRNAs and vsiRNA-based antiviral immunity in diverse plants (Akbergenov et al. 2006; Mlotshwa et al. 2008; Raja et al. 2008; Garcia-Ruiz et al. 2010; Wang et al. 2010; Duan et al. 2012; Raja et al. 2014; Parent et al. 2015).

Plants use different RNA silencing pathways to respond to various types of virus. dsRNA viruses can be directly targeted by dicer-like protein (DCL) enzymes to form vsiRNAs, while single-stranded RNA (ssRNA) viruses require RDRs to form dsRNAs, which are then recognized by DCLs (Brodersen and Voinnet 2006) (Figure 1B). Furthermore, dsRNA structures generated during the replication of ssRNA viruses can also be targeted by DCLs to form vsiRNAs (Brodersen and Voinnet 2006). DCL2 and DCL4, as well as RDR1 and RDR6, are shown to function in antiviral defense. The *dcl2*, *dcl4*, *rdr1* and *rdr6* mutants exhibited significant reduction of vsiRNAs, which suggests they have important roles in vsiRNA biosynthesis (Deleris et al. 2006; Qi et al. 2009). Distinct from defense against RNA viruses, plants infected with DNA viruses usually

undergo genome methylation as an epigenetic defense. PolIV, PolV, RDR2, dsRNA binding protein 3 (DRB3), and DCL3 are involved in the biogenesis of 24-nt vsiRNAs (Pikaard et al. 2012; Raja et al. 2014), which induce TGS by directing DNA methylation (Ding 2010; Melnyk et al. 2011a). These vsiRNAs subsequently load into AGO4 and participate in a RNA-directed DNA methylation (RdDM) pathway. To achieve effective RNA-based antiviral immunity, vsiRNAs are also amplified by RDRs to produce secondary siRNAs (Garcia-Ruiz et al. 2010; Wang et al. 2010; Wang et al. 2011). In addition to simply increasing vsiRNA accumulation, the secondary vsiRNAs are also able to target regions of mRNAs not targeted by the primary vsiRNAs. RDR1/RDR6 were shown to synthesize *de novo* dsRNAs using cleaved viral mRNA as templates, and the new dsRNA are further processed by DCLs to produce secondary vsiRNAs (Ding 2010). However, in *N. benthamiana*, despite its activity in antiviral resistance mediated by salicylic acid, RDR1 appears to suppress RDR6-mediated antiviral RNA silencing (Ying et al. 2010).

Plant miRNAs have also shown to have a profound impact on defense against viruses. The accumulation of miRNAs is significantly affected by viral infection. In rice, the expression patterns of 14 miRNAs in leaves and 16 miRNAs in roots changed significantly in response to Rice black streaked dwarf virus (RBSDV) infection (Sun et al. 2015). In tomato (*Solanum lycopersicum*), the expression of 53 novel miRNAs were shown to undergo changes in response to Tomato leaf curl New Delhi virus (ToLCNDV) infection (Pradhan et al. 2015). Rice stripe virus (RSV, a negative sense and ambisense RNA virus) increased the titer of some rice miRNAs and phasiRNAs, while Rice dwarf virus (RDV, a dsRNA virus) did not show significant effect on rice sRNA expression (Du et al. 2011). Furthermore, some miRNAs appear to target *R* genes to regulate resistance against viruses. In tobacco, miR482 was shown to target mRNAs for NBS-LRR proteins, causing mRNA decay and the production of phasiRNAs. Tomato plants infected with Turnip crinkle virus (TCV), Cucumber mosaic virus (CMV), and Tobacco rattle virus (TRV) showed a suppressed miR482-mediated silencing cascade, resulting in increased expression of miR482-targeted mRNA (Shivaprasad et al. 2012). A similar result was also observed in tobacco infected with Tobacco mosaic virus (TMV). miR6019 and miR6020 in tobacco confers resistance through the regulation of NSB-LRRs and the production of secondary 21-nt siRNAs (Li et al. 2012). In response to infection with RSV and RDV, miR168 was sequestered to alleviate its repression on rice AGO1 to confer broad-spectrum viral resistance (Wu et al. 2015).

Viral defense is achieved not only by inhibiting viral replication within the cell but also restricting cell-to-cell viral movement. In higher plants, viral immunity mediated by sRNAs is not limited to the infected cells but can spread and silence viral RNAs in distant tissue (Palauqui et al. 1997). Tobacco RDR6 has been shown to be involved in defense against viruses at the level of systemic spreading, most likely through the generation of secondary siRNAs (Schwach et al. 2005). It was later shown that 21-nt and 24-nt siRNA move between cells or in long-range transport through the phloem (Dunoyer et al. 2010; Molnar et al. 2010; Melnyk et al. 2011a). Both 21-nt and 24-nt siRNA duplexes, but not ssRNAs, can move in short distances. However, only 21-nt siRNA duplexes are required for the silencing spreading (Dunoyer et al. 2010). In long-range transport, although both 21-nt and 24-nt siRNAs can move within the phloem, only 24-nt siRNAs can spread the silencing (Molnar et al. 2010; Melnyk et al. 2011a). Thus, both 21-nt and 24-nt siRNAs are important for the signal transport. The function of the mobile siRNAs most likely depends on the RNAi silencing components in the recipient tissue rather than on the mobility of siRNAs (Sarkies and Miska 2014).

Plants developed sRNA-based gene silencing as a defense strategy against viruses and other pathogens. To counteract this defense strategy, viruses have evolved specific proteins, called viral suppressors of RNA silencing (VSRs), to suppress RNA silencing. More than 80 VSRs have been identified from around 110 plant viruses (Csorba et al. 2015). These VSRs mainly target vsiRNAs, the proteins involved in RNA silencing pathways, such as RDR, DCL and AGO for function (Ding and Voinnet 2007; Burgyan and Havelda 2011; Csorba et al. 2015). In addition to VSRs, viruses have also developed other means to escape host defense mechanisms. vsiRNAs, which are generated by host plants to resist viral infection, can also silence the host genes, such as *CPSF30* and a protein similar to TRAPa, through shared sequence identity to facilitate viral pathogenicity and replication (Qi et al. 2009). Viruses also encode miRNAs that target specific host genes and pathways to enhance their infectivity and/or proliferation (Zhuo et al. 2013). However, there are very few studies on virus-encoded miRNAs in plants, and their functions remain unknown (Gao et al. 2012; Zhuo et al. 2013). Furthermore, some forms of the viral genome, such as defective interfering (DI) RNAs and satellite RNAs, can produce specific siRNAs and suppressors, which regulate host genes, induce specific symptoms and stabilize virus RNAs. For instance, CMV Y satellite RNA (Y-sat) produces specific siRNAs, which down-regulate the mRNA level of *Chli* (a key gene involved in chlorophyll synthesis) and cause bright yellow symptoms (Shimura et al. 2011; Smith et al. 2011). Betasatellites in begomoviruses is necessary for the elicitation of disease symptoms (Qazi et al. 2007). The βC1 protein encoded by a betasatellite interferes with DNA methylation by interacting with S-adenosyl homocysteine hydrolase (SAHH), a methyl cycle enzyme required for TGS (Yang et al. 2011). Meanwhile, βC1 can suppress PTGS by up-regulating *N. benthamiana* calmodulin-like protein (Nbrgs-CaM) (Li et al. 2014a).

sRNAs facilitate plant immunity against insect herbivores

More than one million insects obtain nutrients from plants. To defend against insect pest attack, plants have several physical barriers in place, such as trichomes, hairs and wax (Kessler and Baldwin 2002; Howe and Jander 2008). Plant hormone levels are also altered during insect herbivore attacks. The changes in plant hormone signaling gene expression result in the accumulation of phytochemicals, which can be toxic to insect herbivores. In addition to plant hormones, increasing evidence suggests that plant RNAi machinery plays essential roles in plant immunity against insect herbivores (Figure 1C). The silencing of RDR1 in coyote tobacco (*Nicotiana attenuate*) significantly increased plant susceptibility to a moth *Manduca sexta*, which suggested sRNAs may be involved in plant defense against insect herbivores (Pandey and Baldwin 2007). Further experiments supported this. *M. sexta* attack could be mimicked by the application of larval oral secretions (OS) to puncture wounds. The *N. attenuata* transcriptome of sRNAs

was profiled before and after OS elicitation in wild-type (WT) and *rdr1*-silenced plants. Results showed OS elicitation results in the up-regulation and down-regulation of numerous sRNAs, which may correspond to the large-scale transcriptional changes that occur after herbivore attack (Pandey et al. 2008).

Aphid is one of the important insect herbivores that cause significant crop lost both by feeding on photo assimilates and transmitting many devastating plant viruses (Smith and Boyko 2007). The co-evolution of plants and aphids seems to follow the plant-pathogen arms-race model. Host surface-localized PRRs have been shown to play a role in aphid resistance. Extracts from the green peach aphid (*Myzus persicae*) triggers a PTI-like response in *Arabidopsis* (Prince et al. 2014). In addition, the effectors secreted by aphids may be recognized by R proteins and trigger ETI. Insect attack results in the regulation of hormones (Kerchev et al. 2013), resistance genes and secondary metabolites in plants (Rossi et al. 1998; Kettles et al. 2013). An active interplay between plant and aphids has been studied at a molecular level. Aphid feeding on *Arabidopsis* was found to cause the conversion of one indole glucosinolate to another in plants to boost plant defense (Kim and Jander 2007). Another secondary metabolite found to contribute to defense response against aphid was camalexin. Aphids exposed to camalexin produce less progeny, whereas more progeny was produced by aphid feeding on camalexin-defective plants (Kettles et al. 2013). Plant hormone levels are changed upon aphid feeding. For instance, in response to infestation with *M. persicae*, *Arabidopsis* was shown to modify its hormone level by inducing the signaling and biosynthesis genes for SA, ethylene (ET), and ABA and repressing JA-responsive genes (Kerchev et al. 2013). Interestingly, the *Arabidopsis* miRNA pathway is also involved in camalexin-related aphid resistance. Upon exposure to aphids, miRNA pathway mutants (*dcl1*) showed a significant increase in the transcript level of *PAD3*, a marker for the camalexin biosynthetic pathway. Meanwhile, aphid fecundity was reduced in miRNA pathway mutants but not in mutants defective in siRNA pathways (Kettles et al. 2013). Aphids attack plants by injecting their specialized mouthpart (stylets) into the phloem to suck nutrients. During feeding, aphids secrete saliva as soon as the plasma membrane of the plant cell is punctured. In addition, the honeydew secreted by aphids may also contain molecules that alter plant defense responses. These steps allow aphids to transport viruses and/or aphid effectors into the plant (Jaouannet et al. 2014). However, whether insect herbivores generate dsRNAs/sRNAs that deliver into plants and regulate plant immunity responses remains a question to be answered.

CONVERSATION ACROSS KINGDOMS VIA sRNA

The role of sRNAs in plant immunity suggests there is communication between a plant host and its attacker. Recently, this communication has been observed in various plant-pathogen/insect/parasite/symbiotic interactions (Baum et al. 2007; Mao et al. 2007; Nowara et al. 2010; Helber et al. 2011; Ibrahim et al. 2011; Koch et al. 2013; Weiberg et al. 2013). In plants, sRNAs can move from cell to cell through plasmodesmata (PD) and long distances through phloem vascular tissue (Molnar et al. 2010; Brosnan and Voinnet 2011). Transgenic plants that express exogenous sRNAs/dsRNAs can successfully trigger the silence of genes in pathogens and pests, suggesting plants are able to transfer sRNAs as silencing information to the interacting organisms (Melnyk et al. 2011b; Molnar et al. 2011; Mittelbrunn and Sanchez-Madrid 2012). However, the mechanism of sRNA transport between plant and pathogens is unclear.

It has been established that sRNAs can be delivered into animal cells though vesicular and non-vesicular transportation. Vesicular transportation involves the sorting of sRNAs into vesicles via exosomes and the fusion of exosomes to the plasma membrane to release the sRNAs (Knip et al. 2014; Weiberg et al. 2015). Non-vesicular transportation refers to the direct uptake of environmental RNA signals through RNA transporters. Two membrane-associated RNA transporters, systemic RNAi defective-1 (SID1) and SID2, were identified in *C. elegans*, which are responsible for the uptake of dsRNA into cells (Shih and Hunter 2011; McEwan et al. 2012). During the interaction between fungi and plants, specialized cells called haustorium are secreted by some fungi to form an interface (Duan et al. 2012). Many activities occur at this interface, including uptake of nutrients, delivery of enzymes and toxin into plant cells, secretion of fungal effector protein, and biogenesis of cell surface sensors. It is possible that the transport of sRNAs also occurs at this surface. Koch and Kogel (2014) suggested that sRNAs translocate from plant cytoplasm to haustorium via exosomes, and this movement may require membrane-associated receptors. However, no membrane-associated RNA transporters have yet been identified in plant pathogens.

The transfer of sRNAs is not limited to the host-to-pathogen direction, but rather is bi-directional transportation (Weiberg et al. 2015). *B. cinerea* (Bc), an aggressive fungal pathogen, has been shown to transport Bc-sRNAs into the plants and hijack the host RNAi machinery (Weiberg et al. 2013). After infection of *B. cinerea* on *Arabidopsis* and tomato (*Solanum lycopersicum*), a total of 832 Bc-RNAs were found in both *Arabidopsis* and tomato (Weiberg et al. 2013). In addition, the function of Bc-sRNAs was further characterized by expressing Bc-sRNAs in *Arabidopsis*. Bc-sRNA selectively silences host immunity genes by binding to *Arabidopsis* AGO1. A number of tRNA-derived RNA fragments (19–40-nt) from oomycete *Phytophthora infestans* was also found in infected potato leaves, which suggests the translocation of tRNA-derived sRNAs from pathogen to host plants (Asman et al. 2014). However, the function of tRNA-derived sRNAs has not been characterized. In pathogen-animal interaction, small noncoding RNAs (snRNAs) from the endosymbiotic bacteria, *Wolbachia*, act as effectors to modulate the expression of mosquito host genes (Mayoral et al. 2014). sRNAs were identified in parasite *Leishmania* exosomes, which were eventually taken up by host cells (Lambertz et al. 2015). The animal-parasitic nematode *Heligmosomoides polygyrus* has also been shown to secrete exosomes to transfer miRNAs to mammalian cells (Buck et al. 2014). These studies suggest that a cross-kingdom RNAi machinery may exist as an advanced virulence mechanism.

THE APPLICATION OF sRNAS IN PLANT PROTECTION

RNA silencing-mediated pathogen-derived resistance

Pests and pathogens are two major sources of biotic stress limiting plant growth and development (Howe and Jander 2008; Atkinson and Urwin 2012). In the battle of survival, plants developed sRNAs to silence particular genes to protect themselves from pathogen attack (Wingard 1928). A decade before the identification of RNA silencing, Sanford and Johnson proposed the concept of parasite/pathogen-derived resistance (PDR) by transforming a pathogen gene fragment into the plant or animal host (Sanford and Johnston 1985). Scientists utilized the properties of RNA silencing and developed a strategy, to create plants with increased resistance against pathogen and insect herbivores. PDR was first widely used in antiviral resistance and the most successful case was transgenic papaya resistant to Papaya ringspot virus (PSRV) (Sanford and Johnston 1985; Baulcombe 1996; Gonsalves 1998). With the discovery of RNA silencing, transgenic plants that express exogenous RNAi targeting essential genes in pathogens and insect herbivores have been developed to protect plants from many pathogens and pests.

To produce effective PDR, the source of the sRNA precursor is critical, as the source is closely correlated with silencing efficacy (Duan et al. 2012; Nunes and Dean 2012). For instance, sRNAs generated from hairpin constructs more effectively silenced GFP than those derived from sense and antisense constructs (Kadotani et al. 2003); transgenic plants expressing artificial miRNAs (amiRNAs) were more efficient in silencing the same target gene and also increased insect herbivore resistance compared to plants expressing hairpin RNAs (hpRNAs) (Guo et al. 2014). In addition to silencing efficiency, off-target effects and the persistence of sRNAs are considerations in sRNA selection. It is necessary to ensure that PDR constructs do not target and negatively affect the host. Meanwhile, the selection of target genes is also very important. Promising PDR targets should be genes that are critical for the development and growth of pathogens, or play important roles in the plant-pathogen interaction.

PDR has been successfully applied in various plants, including model plants, such as *Arabidopsis* and tobacco *N. benthamiana*, and important crops, such as rice, maize, cotton, wheat and barley (Nunes and Dean 2012; Koch and Kogel 2014). Transgenic plants that target the viral DNA/RNA genome or virus proteins were generated before the demonstration of the RNAi mechanism (Abel et al. 1986; Duan et al. 2012). Viral transgene-derived siRNAs, viral-derived hpRNAs and artificial miRNAs were manipulated and expressed in transgenic plants to induce RNAi to increase antiviral resistance (Kawchuk et al. 1990; Canto and Palukaitis 1998; Chellappan et al. 2004; Fagoaga et al. 2006; Ai et al. 2011; Duan et al. 2012). amiRNAs that contain the sequence of suppressor 2b of CMV efficiently inhibit 2b gene expression and improve plant resistance to CMV (Qu et al. 2007). Plants transformed with amiRNAs targeting V2 genes in Cotton leaf curl Burewala virus (CLCuBuV) also showed increased resistance against CLCuBuV (Ali et al. 2013).

Unlike plant viruses, which replicate inside infected plant cells, PDR against other pathogens require the transfer of sRNAs from host to pathogen. Fortunately, the cross-talk between plant and pathogen via sRNAs allows the application of PDR in other pathogens (Weiberg et al. 2015). PDR also functions in controlling fungal pathogen infection. PDR to fungi, which is termed host-induced gene silencing (HIGS), has been shown in barley and wheat against fungal pathogen *B. graminis* (Nowara et al. 2010). Transgenic expression of dsRNAs targeting fungal glucanosyl transferase genes or fungal effector gene *Avral10* (in the absence of *Mal10*) reduces the formation of haustoria and thus increases resistance to *B. graminis*. Other pathogenicity genes, such as cytochrome P450 lanosterol C14α-demethylase (CYP51A1)-encoding genes and Foc race 1 *velvet* in *Fusarium*, and genes encoding a mitogen-activated protein (MAP) kinase, a cyclophilin, and a calcineurin B in *Pucciniatriticina* (Pt), were chosen as HIGS targets, and their inhibition resulted in suppression of fungal growth (Koch et al. 2013; Panwar et al. 2013; Ghag et al. 2014).

In addition to defense against pathogens, mechanisms similar to PDS have been used as a useful tool in insect control. *Bacillus thuringiensis* insecticidal proteins are widely used to control the Lepidopteran and Coleopteran insect pest (James 2003; Vaughn et al. 2005). However, the emergence of insect herbivore resistance to transgenic plants over-expressing these *Bacillus thuringiensis* proteins requires us to develop a new method to control insect herbivores. Many insect genes can be silenced by injection or oral administration of dsRNAs (Figure 1C). Thus, transgenic plants expressing dsRNAs targeted essential insect genes were generated to be resistant against insect herbivore attack. Studies on the moth cotton bollworm (*Helicoverpa armigera*) revealed that plants expressing *CYP6AE14* dsRNAs triggered RNAi in the moth midgut, which suppressed the expression of *CYP6AE14* and retarded larval growth (Mao et al. 2007; Mao et al. 2011). Cotton plants expressing the *CYP6AE14* dsRNAs in addition to plant cysteine proteases, which increased permeability to the midgut, exhibited increased resistance to the moth (Mao et al. 2013). Moreover, feeding insects with dsRNAs supplied in an artificial diet resulted in the down-regulation of target genes in several coleopteran (beetle) species. Transgenic corn plants engineered to express *dsV-ATPaseA* showed a higher resistance to western corn rootworm (*Diabrotica virgifera virgifera*) and caused growth inhibition and mortality of the insect herbivores (Baum et al. 2007). Feeding western corn rootworm dsRNAs 60 bp or longer also triggered the silencing of the target *DvSnf7* and resulted in increased larvae mortality (Bolognesi et al. 2012). A recent study shows that long dsRNAs, but not siRNAs, can be uptaken by the midgut cells of western corn rootworm and Colorado potato beetle. The long dsRNAs were subsequently processed into 21-nt siRNAs by various insect herbivores and accumulated in distal cells to regulate gene expression (Ivashuta et al. 2015). These results suggest plants can produce dsRNAs or sRNAs to resist insect herbivore attack, and RNAi can be utilized to reduce insect herbivore damage.

Insect herbivores that feed on transgenic plants carrying RNAi constructs have shown reduced growth, decreased reproduction rate and increased susceptibility to insecticides (Baum et al. 2007; Mao et al. 2007; Pitino et al. 2011; Tao et al. 2012; Xu et al. 2014). Although this method was successfully applied in many plants against insect herbivores, it has been difficult to generate transgenic plants that produce dsRNA that stably and permanently target insect genes. The DCL

proteins in the plant prevent the accumulation of a high number of long dsRNAs by processing dsRNAs into siRNAs. Recently, Zhang et al. (2015) developed an efficient pest control system by producing dsRNA in chloroplasts, a cellular organelle that appears to lack a RNA pathway. Colorado potato beetles (*Leptinotarsa decemlineata*) which fed on transgenic potato plants producing dsRNAs in chloroplasts showed a significantly high rate of lethality.

Although PDS has been successfully applied to several plants, there are still limitations in utilizing this strategy for plant protection. sRNA-mediated silencing efficacy can be affected by many factors, including pathogen type, pathogen titer and environment stress. The successful application of PDR in plants growing in greenhouses cannot fully model the effect in the field. Since mixed infections are common in nature, PDR may need to target genes in multiple pathogens. The tolerance of transgenic plants to environment stresses, such as temperature, drought, and salinity should also be considered. Further research is required to solve these problems.

Transgenerational defense in plant biotic stress response

Increasing evidence has shown that pathogen attack on plants can induce a particular defense response which can be passed on to the offspring, a term called priming. For instance, the progeny of plants infected with TMV showed an increase in homologous recombination frequency (HRF), PR1 expression, callose deposition and also resistance to TMV (Kathiria et al. 2010). Moreover, the increased resistance in the progeny generation is not only against the virus but also against bacteria (*P. syringae*) and oomycete (*P. nicotianae*) (Kathiria et al. 2010). dsDNA virus CaMV also induced transgenerational defense in rapeseed (*Brassica napus*) (Kalischuk et al. 2015). In addition to plant-virus interaction, transgenerational induction of defense was also observed in *Arabidopsis* treated with *Pst* DC3000 carrying effector gene *avrRpt2*. *Pst avrRpt2* enhanced resistance in the next generation to both *P. syringae* and the oomycete *Hyaloperonospora arabidopsidis* (Slaughter et al. 2012). In another study, the increased resistance was sustained even over one infection-free generation (Luna et al. 2012). Transgenerational defense is not limited to the pathogen stress, but also to insect herbivores. Herbivore damage to the wild radish (*Raphanus raphanistrum*) induced transgenerational defense, which resulted in the production of radish offspring with higher resistance (Agrawal et al. 1999). The demonstration of increased trichome production in the offspring of leaf-damaged yellow monkey flower provides another evidence for transgenerational defense (Holeski 2007; Scoville et al. 2011). In addition, caterpillar herbivory on *Arabidopsis* and tomato induced transgenerational resistance in both species, manifested as the retarded growth of the caterpillar (Rasmann et al. 2012).

Recent studies show that transgenerational resistance triggered by pathogen/insect attack is passed on to the offspring through DNA methylation, sRNA accumulation or histone modification. Luna et al. (2012) generated *Arabidopsis* progeny (P1) with transgenerational resistance by infecting the parents with *Pst* DC3000. *Arabidopsis* mutants defective in three DNA methyltransferases *drm1 drm2 cmt3* (*ddc*), displayed the same resistance phenotype to *H. arabidopsidis* as P1. The hypomethylated DNA in the *ddc* mutant mimics the transgenerational resistance phenotype of progeny, indicating that transgenerational resistance induced by *Pst* DC3000 is transmitted by hypomethylated DNA (Luna et al. 2012). In this form of inheritance of resistance, sRNAs appear to play an essential role by mediating the process of RdDM. This theory is supported by the finding that transgenerational resistance to caterpillar was abolished in *Arabidopsis nrpd2a/nrpd2b* and *dcl2/dcl3/dcl4* mutants, which are deficient in hc-siRNA synthesis and processing (Rasmann et al. 2012). Another study demonstrated that sRNA can guide genome reprogramming in pollen. 24-nt siRNA-guided *de novo* DNA methyltransferase can restore CHH methylation in microspores and sperm cells (Calarco et al. 2012). The active DNA methyltransferases guided by hc-siRNAs during gametogenesis and embryogenesis allow the resistance to pass down from parent to progeny (Blevins et al. 2014). Many proteins that are involved in the biogenesis of sRNA or RdDM are also shown to be involved in epigenetic inheritance (Saze and Kakutani 2007; Nuthikattu et al. 2013; Zhong et al. 2013). Holeski et al. (2012) described a model of transgenerational induction: in response to environmental cues, chemical and physical defenses are induced, and phloem-mobile sRNAs move from vegetative tissue to developing seeds as a form of stored information to be passed on to the next generation (Holeski et al. 2012). However, this hypothesis that sRNAs carry information for transgenerational defense has not yet been confirmed.

CONCLUSIONS

There is constant resource competition between plant defense and growth. The plant immune system protects them from pathogen attack, but it also competes for the limited resources available for plant growth and development. Thus, plant immunity is a complex and highly regulated system. Numerous miRNAs and siRNAs are present in plants, which play essential roles in plant growth, development and immunity. In response to biotic and abiotic stress, sRNAs fine-tune the expression of plant hormones and resistance genes to achieve the balance between defense and growth. The important roles of sRNAs have attracted many researchers to investigate the biogenesis, mode of action, and the target of sRNAs which are particularly involved during plant-pathogen/insect herbivore interaction. In addition, the basic research on sRNA has provided information for scientists to utilize the features of sRNA and generate transgenic plants with disease and insect herbivore resistance. Of course, there are still many challenges in applying these techniques in the field. Further study on sRNAs as well as their function in transgenic plants would provide a powerful tool to protect plants from pathogen and insect herbivore attack and also improve food production.

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REFERENCES

Abel PP, Nelson RS, De B, Hoffmann N, Rogers SG, Fraley RT, Beachy RN (1986) Delay of disease development in transgenic plants that express the tobacco mosaic-virus coat protein gene. **Science** 232: 738–743

Adkar-Purushothama CR, Brosseau C, Giguere T, Sano T, Moffett P, Perreault JP (2015) Small RNA derived from the virulence modulating region of the potato spindle tuber viroid silences callose synthase genes of tomato plants. **Plant Cell** 27: 2178–2194

Agrawal AA, Laforsch C, Tollrian R (1999) Transgenerational induction of defences in animals and plants. **Nature** 401: 60–63

Ai T, Zhang L, Gao Z, Zhu CX, Guo X (2011) Highly efficient virus resistance mediated by artificial microRNAs that target the suppressor of PVX and PVY in plants. **Plant Biol** 13: 304–316

Akbergenov R, Si-Ammour A, Blevins T, Amin I, Kutter C, Vanderschuren H, Zhang P, Gruissem W, Meins F, Jr, Hohn T, Pooggin MM (2006) Molecular characterization of geminivirus-derived small RNAs in different plant species. **Nucleic Acids Res** 34: 462–471

Ali I, Amin I, Briddon RW, Mansoor S (2013) Artificial microRNA-mediated resistance against the monopartite begomovirus *Cotton leaf curl Burewala virus*. **Virol J** 10: 231

Asman AK, Vetukuri RR, Jahan SN, Fogelqvist J, Corcoran P, Avrova AO, Whisson SC, Dixelius C (2014) Fragmentation of tRNA in *Phytophthora infestans* asexual life cycle stages and during host plant infection. **BMC Microbiol** 14: 308

Atkinson NJ, Urwin PE (2012) The interaction of plant biotic and abiotic stresses: From genes to the field. **J Exp Bot** 63: 3523–3543

Avina-Padilla K, de la Vega OM, Rivera-Bustamante R, Martinez-Soriano JP, Owens RA, Hammond RW, Vielle-Calzada JP (2015) In silico prediction and validation of potential gene targets for pospiviroid-derived small RNAs during tomato infection. **Gene** 564: 197–205

Bartel DP (2009) MicroRNAs: Target recognition and regulatory functions. **Cell** 136: 215–233

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

Baulcombe DC (1996) Mechanisms of pathogen-derived resistance to viruses in transgenic plants. **Plant Cell** 8: 1833–1844

Baum JA, Bogaert T, Clinton W, Heck GR, Feldmann P, Ilagan O, Johnson S, Plaetinck G, Munyikwa T, Pleau M, Vaughn T, Roberts J (2007) Control of coleopteran insect pests through RNA interference. **Nat Biotechnol** 25: 1322–1326

Bebber DP, Gurr SJ (2015) Crop-destroying fungal and oomycete pathogens challenge food security. **Fungal Genet Biol** 74: 62–64

Bian H, Xie Y, Guo F, Han N, Ma S, Zeng Z, Wang J, Yang Y, Zhu M (2012) Distinctive expression patterns and roles of the miRNA393/TIR1 homolog module in regulating flag leaf inclination and primary and crown root growth in rice (*Oryza sativa*). **New Phytol** 196: 149–161

Bigeard J, Colcombet J, Hirt H (2015) Signaling mechanisms in pattern-triggered immunity (PTI). **Mol Plant** 8: 521–539

Blevins T, Pontvianne F, Cocklin R, Podicheti R, Chandrasekhara C, Yerneni S, Braun C, Lee B, Rusch D, Mockaitis K, Tang H, Pikaard CS (2014) A two-step process for epigenetic inheritance in Arabidopsis. **Mol Cell** 54: 30–42

Boccara M, Sarazin A, Thiebeauld O, Jay F, Voinnet O, Navarro L, Colot V (2014) The Arabidopsis *miR472-RDR6* silencing pathway modulates PAMP- and effector-triggered immunity through the post-transcriptional control of disease resistance genes. **PLoS Pathog** 10: e1003883

Bolognesi R, Ramaseshadri P, Anderson J, Bachman P, Clinton W, Flannagan R, Ilagan O, Lawrence C, Levine S, Moar W, Mueller G, Tan J, Uffman J, Wiggins E, Heck G, Segers G (2012) Characterizing the mechanism of action of double-stranded RNA activity against western corn rootworm (*Diabrotica virgifera virgifera* LeConte). **PLoS ONE** 7: e47534

Brodersen P, Voinnet O (2006) The diversity of RNA silencing pathways in plants. **Trends Genet** 22: 268–280

Brosnan CA, Voinnet O (2011) Cell-to-cell and long-distance siRNA movement in plants: Mechanisms and biological implications. **Curr Opin Plant Biol** 14: 580–587

Buck AH, Coakley G, Simbari F, McSorley HJ, Quintana JF, Le Bihan T, Kumar S, Abreu-Goodger C, Lear M, Harcus Y, Ceroni A, Babayan SA, Blaxter M, Ivens A, Maizels RM (2014) Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity. **Nat Commun** 5: 5488

Burgyan J, Havelda Z (2011) Viral suppressors of RNA silencing. **Trends Plant Sci** 16: 265–272

Calarco JP, Borges F, Donoghue MT, Van Ex F, Jullien PE, Lopes T, Gardner R, Berger F, Feijo JA, Becker JD, Martienssen RA (2012) Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA. **Cell** 151: 194–205

Campo S, Peris-Peris C, Sire C, Moreno AB, Donaire L, Zytnicki M, Notredame C, Llave C, San Segundo B (2013) Identification of a novel microRNA (miRNA) from rice that targets an alternatively spliced transcript of the *Nramp6* (*Natural resistance-associated macrophage protein 6*) gene involved in pathogen resistance. **New Phytologist** 199: 212–227

Canto T, Palukaitis P (1998) Transgenically expressed cucumber mosaic virus RNA 1 simultaneously complements replication of cucumber mosaic virus RNAs 2 and 3 and confers resistance to systemic infection. **Virology** 250: 325–336

Cao Y, Liang Y, Tanaka K, Nguyen CT, Jedrzejczak RP, Joachimiak A, Stacey G (2014) The kinase LYK5 is a major chitin receptor in *Arabidopsis* and forms a chitin-induced complex with related kinase CERK1. **Elife** 3: 03766

Chapman EJ, Carrington JC (2007) Specialization and evolution of endogenous small RNA pathways. **Nat Rev Genet** 8: 884–896

Chellappan P, Masona MV, Vanitharani R, Taylor NJ, Fauquet CM (2004) Broad spectrum resistance to ssDNA viruses associated with transgene-induced gene silencing in cassava. **Plant Mol Biol** 56: 601–611

Chen L, Luan Y, Zhai J (2015) Sp-miR396a-5p acts as a stress-responsive genes regulator by conferring tolerance to abiotic stresses and susceptibility to *Phytophthora Nicotianae* infection in transgenic tobacco. **Plant Cell Rep**: 1–13

Chen XM (2009) Small RNAs and their roles in plant development. **Annu Rev Cell Dev Biol** 25: 21–44

Csorba T, Kontra L, Burgyan J (2015) Viral silencing suppressors: Tools forged to fine-tune host-pathogen coexistence. **Virology** 479–480: 85–103

Cui X, Cao X (2014) Epigenetic regulation and functional exaptation of transposable elements in higher plants. **Curr Opin Plant Biol** 21: 83–88

Deleris A, Gallego-Bartolome J, Bao J, Kasschau KD, Carrington JC, Voinnet O (2006) Hierarchical action and inhibition of plant Dicer-like proteins in antiviral defense. **Science** 313: 68–71

Ding SW (2010) RNA-based antiviral immunity. **Nat Rev Immunol** 10: 632–644

Ding SW, Voinnet O (2007) Antiviral immunity directed by small RNAs. **Cell** 130: 413–426

Dou D, Zhou JM (2012) Phytopathogen effectors subverting host immunity: Different foes, similar battleground. **Cell Host Microbe** 12: 484–495

Du P, Wu J, Zhang J, Zhao S, Zheng H, Gao G, Wei L, Li Y (2011) Viral infection induces expression of novel phased microRNAs from conserved cellular microRNA precursors. **PLoS Pathog** 7: e1002176

Duan CG, Wang CH, Guo HS (2012) Application of RNA silencing to plant disease resistance. **Silence** 3: 5

Dunoyer P, Schott G, Himber C, Meyer D, Takeda A, Carrington JC, Voinnet O (2010) Small RNA duplexes function as mobile silencing signals between plant cells. **Science** 328: 912–916

Fagoaga C, Lopez C, de Mendoza AH, Moreno P, Navarro L, Flores R, Pena L (2006) Post-transcriptional gene silencing of the p23 silencing suppressor of Citrus tristeza virus confers resistance to the virus in transgenic Mexican lime. **Plant Mol Biol** 60: 153–165

Fahlgren N, Howell M, Kasschau K, Chapman E, Sullivan C, Cumbie J, Givan S, Law T, Grant S, Dangl J, Carrington J (2007) High-throughput sequencing of *Arabidopsis* microRNAs: Evidence for frequent birth and death of *MIRNA* genes. **PLoS ONE** 2: e219

Feng F, Zhou JM (2012) Plant-bacterial pathogen interactions mediated by type III effectors. **Curr Opin Plant Biol** 15: 469–476

Feng H, Zhang Q, Wang Q, Wang X, Liu J, Li M, Huang L, Kang Z (2013) Target of tae-miR408, a chemocyanin-like protein gene (*TaCLP1*), plays positive roles in wheat response to high-salinity, heavy cupric stress and stripe rust. **Plant Mol Biol** 83: 433–443

Flood J (2010) The importance of plant health to food security. **Food Security** 2: 215–231

Gao R, Liu P, Wong SM (2012) Identification of a plant viral RNA genome in the nucleus. **PLoS ONE** 7: e48736

Garcia-Ruiz H, Takeda A, Chapman EJ, Sullivan CM, Fahlgren N, Brempelis KJ, Carrington JC (2010) *Arabidopsis* RNA-dependent RNA polymerases and Dicer-Like proteins in antiviral defense and small Interfering RNA biogenesis during *turnip mosaic virus* infection. **Plant Cell** 22: 481–496

Ghag SB, Shekhawat UK, Ganapathi TR (2014) Host-induced post-transcriptional hairpin RNA-mediated gene silencing of vital fungal genes confers efficient resistance against Fusarium wilt in banana. **Plant Biotechnol J** 12: 541–553

Gomez-Gomez L, Boller T (2000) FLS2: An LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. **Mol Cell** 5: 1003–1011

Gonsalves D (1998) Control of papaya ringspot virus in papaya: A case study. **Annu Rev Phytopathol** 36: 415–437

Guo H, Song X, Wang G, Yang K, Wang Y, Niu L, Chen X, Fang R (2014) Plant-generated artificial small RNAs mediated aphid resistance. **PLoS ONE** 9: e97410

Guo N, Ye WW, Wu XL, Shen DY, Wang YC, Xing H, Dou DL (2011) Microarray profiling reveals microRNAs involving soybean resistance to *Phytophthora sojae*. **Genome** 54: 954–958

Gupta OP, Permar V, Koundal V, Singh UD, Praveen S (2012) MicroRNA regulated defense responses in *Triticum aestivum* L. during *Triticum aestivum* f.sp. *tritici* infection. **Mol Biol Rep** 39: 817–824

Hamilton AJ, Baulcombe DC (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. **Science** 286: 950–952

Helber N, Wippel K, Sauer N, Schaarschmidt S, Hause B, Requena N (2011) A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus *Glomus* sp is crucial for the symbiotic relationship with plants. **Plant Cell** 23: 3812–3823

Holeski LM (2007) Within and between generation phenotypic plasticity in trichome density of *Mimulus guttatus*. **J Evol Biol** 20: 2092–2100

Holeski LM, Jander G, Agrawal AA (2012) Transgenerational defense induction and epigenetic inheritance in plants. **Trends Ecol Evol** 27: 618–626

Howe GA, Jander G (2008) Plant immunity to insect herbivores. **Annu Rev Plant Biol** 59: 41–66

Ibrahim HM, Alkharouf NW, Meyer SL, Aly MA, Gamal El-Din Ael K, Hussein EH, Matthews BF (2011) Post-transcriptional gene silencing of root-knot nematode in transformed soybean roots. **Exp Parasitol** 127: 90–99

Ivashuta S, Zhang Y, Wiggins BE, Ramaseshadri P, Segers GC, Johnson S, Meyer SE, Kerstetter RA, McNulty BC, Bolognesi R, Heck GR (2015) Environmental RNAi in herbivorous insects. **RNA** 21: 840–850

Jagadeeswaran G, Saini A, Sunkar R (2009) Biotic and abiotic stress down-regulate miR398 expression in *Arabidopsis*. **Planta** 229: 1009–1014

James C (2003) Global review of commercialized transgenic crops. **Curr Sci** 84: 303–309

Jaouannet M, Rodriguez PA, Thorpe P, Lenoir CJ, MacLeod R, Escudero-Martinez C, Bos JI (2014) Plant immunity in plant-aphid interactions. **Front Plant Sci** 5: 663

Jones JD, Dangl JL (2006) The plant immune system. **Nature** 444: 323–329

Kadotani N, Nakayashiki H, Tosa Y, Mayama S (2003) RNA silencing in the phytopathogenic fungus *Magnaporthe oryzae*. **Mol Plant Microbe Interact** 16: 769–776

Kalischuk ML, Johnson D, Kawchuk LM (2015) Priming with a double-stranded DNA virus alters *Brassica rapa* seed architecture and facilitates a defense response. **Gene** 557: 130–137

Kathiria P, Sidler C, Golubov A, Kalischuk M, Kawchuk LM, Kovalchuk I (2010) Tobacco mosaic virus infection results in an increase in recombination frequency and resistance to viral, bacterial, and fungal pathogens in the progeny of infected tobacco plants. **Plant Physiol** 153: 1859–1870

Katiyar-Agarwal S, Gao S, Vivian-Smith A, Jin H (2007) A novel class of bacteria-induced small RNAs in *Arabidopsis*. **Gene Dev** 21: 3123–3134

Katiyar-Agarwal S, Jin H (2010) Role of small RNAs in host-microbe interactions. **Annu Rev Phytopathol** 48: 225–246

Katiyar-Agarwal S, Morgan R, Dahlbeck D, Borsani O, Villegas A, Jr, Zhu JK, Staskawicz BJ, Jin H (2006) A pathogen-inducible endogenous siRNA in plant immunity. **Proc Natl Acad Sci USA** 103: 18002–18007

Kawchuk LM, Martin RR, Mcpherson J (1990) Resistance in transgenic potato expressing the potato leafroll virus coat protein gene. **Mol Plant Microbe Interact** 3: 301–307

Kerchev PI, Karpinska B, Morris JA, Hussain A, Verrall SR, Hedley PE, Fenton B, Foyer CH, Hancock RD (2013) Vitamin C and the abscisic acid-insensitive 4 transcription factor are important determinants of aphid resistance in *Arabidopsis*. **Antioxid Redox Signal** 18: 2091–2105

Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: The emerging molecular analysis. **Annu Rev Plant Biol** 53: 299–328

Kettles GJ, Drurey C, Schoonbeek HJ, Maule AJ, Hogenhout SA (2013) Resistance of *Arabidopsis thaliana* to the green peach aphid, *Myzus persicae*, involves camalexin and is regulated by microRNAs. **New Phytol** 198: 1178–1190

Kim JH, Jander G (2007) *Myzus persicae* (green peach aphid) feeding on Arabidopsis induces the formation of a deterrent indole glucosinolate. **Plant J** 49: 1008–1019

Knip M, Constantin ME, Thordal-Christensen H (2014) Trans-kingdom cross-talk: Small RNAs on the move. **PLoS Genet** 10: e1004602

Koch A, Kogel KH (2014) New wind in the sails: Improving the agronomic value of crop plants through RNAi-mediated gene silencing. **Plant Biotechnol J** 12: 821–831

Koch A, Kumar N, Weber L, Keller H, Imani J, Kogel KH (2013) Host-induced gene silencing of cytochrome P450 lanosterol C14α-demethylase-encoding genes confers strong resistance to *Fusarium* species. **Proc Natl Acad Sci USA** 110: 19324–19329

Lambertz U, Oviedo Ovando ME, Vasconcelos EJ, Unrau PJ, Myler PJ, Reiner NE (2015) Small RNAs derived from tRNAs and rRNAs are highly enriched in exosomes from both old and new world Leishmania providing evidence for conserved exosomal RNA Packaging. **BMC Genomics** 16: 151

Li F, Huang C, Li Z, Zhou X (2014a) Suppression of RNA silencing by a plant DNA virus satellite requires a host calmodulin-like protein to repress *RDR6* expression. **PLoS Pathog** 10: e1003921

Li F, Pignatta D, Bendix C, Brunkard JO, Cohn MM, Tung J, Sun H, Kumar P, Baker B (2012) MicroRNA regulation of plant innate immune receptors. **Proc Natl Acad Sci USA** 109: 1790–1795

Li Y, Lu YG, Shi Y, Wu L, Xu YJ, Huang F, Guo XY, Zhang Y, Fan J, Zhao JQ, Zhang HY, Xu PZ, Zhou JM, Wu XJ, Wang PR, Wang WM (2014b) Multiple rice microRNAs are involved in immunity against the blast fungus *Magnaporthe oryzae*. **Plant Physiol** 164: 1077–1092

Li Y, Zhang Q, Zhang J, Wu L, Qi Y, Zhou JM (2010) Identification of microRNAs involved in pathogen-associated molecular pattern-triggered plant innate immunity. **Plant Physiol** 152: 2222–2231

Liu J, Cheng X, Liu D, Xu W, Wise R, Shen QH (2014a) The miR9863 family regulates distinct *Mla alleles* in barley to attenuate NLR receptor-triggered disease resistance and cell-death signaling. **PLoS Genet** 10: e1004755

Liu WD, Liu JL, Triplett L, Leach JE, Wang GL (2014b) Novel insights into rice innate immunity against bacterial and fungal pathogens. **Annu Rev Phytopathol** 52: 213–241

Lozano-Duran R, Bourdais G, He SY, Robatzek S (2014) The bacterial effector HopM1 suppresses PAMP-triggered oxidative burst and stomatal immunity. **New Phytol** 202: 259–269

Luna E, Bruce TJ, Roberts MR, Flors V, Ton J (2012) Next-generation systemic acquired resistance. **Plant Physiol** 158: 844–853

Manavella PA, Koenig D, Weigel D (2012) Plant secondary siRNA production determined by microRNA-duplex structure. **Proc Natl Acad Sci USA** 109: 2461–2466

Mao YB, Cai WJ, Wang JW, Hong GJ, Tao XY, Wang LJ, Huang YP, Chen XY (2007) Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. **Nat Biotechnol** 25: 1307–1313

Mao YB, Tao XY, Xue XY, Wang LJ, Chen XY (2011) Cotton plants expressing *CYP6AE14* double-stranded RNA show enhanced resistance to bollworms. **Transgenic Res** 20: 665–673

Mao YB, Xue XY, Tao XY, Yang CQ, Wang LJ, Chen XY (2013) Cysteine protease enhances plant-mediated bollworm RNA interference. **Plant Mol Biol** 83: 119–129

Mayoral JG, Hussain M, Joubert DA, Iturbe-Ormaetxe I, O'Neill SL, Asgari S (2014) Wolbachia small noncoding RNAs and their role in cross-kingdom communications. **Proc Natl Acad Sci USA** 111: 18721–18726

McEwan DL, Weisman AS, Hunter CP (2012) Uptake of extracellular double-stranded RNA by SID-2. **Mol Cell** 47: 746–754

Melnyk CW, Molnar A, Bassett A, Baulcombe DC (2011a) Mobile 24 nt small RNAs direct transcriptional gene silencing in the root meristems of *Arabidopsis thaliana*. **Curr Biol** 21: 1678–1683

Melnyk CW, Molnar A, Baulcombe DC (2011b) Intercellular and systemic movement of RNA silencing signals. **EMBO J** 30: 3553–3563

Meyers BC, Kaushik S, Nandety RS (2005) Evolving disease resistance genes. **Curr Opin Plant Biol** 8: 129–134

Mittelbrunn M, Sanchez-Madrid F (2012) Intercellular communication: Diverse structures for exchange of genetic information. **Nat Rev Mol Cell Biol** 13: 328–335

Mlotshwa S, Pruss GJ, Peragine A, Endres MW, Li J, Chen X, Poethig RS, Bowman LH, Vance V (2008) *DICER-LIKE2* plays a primary role in transitive silencing of transgenes in *Arabidopsis*. **PLoS ONE** 3: e1755

Moffett P (2009) Mechanisms of recognition in dominant *R* gene mediated resistance. **Adv Virus Res** 75: 1–33

Molnar A, Melnyk C, Baulcombe DC (2011) Silencing signals in plants: A long journey for small RNAs. **Genome Biol** 12: 215

Molnar A, Melnyk CW, Bassett A, Hardcastle TJ, Dunn R, Baulcombe DC (2010) Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. **Science** 328: 872–875

Navarro B, Gisel A, Rodio ME, Delgado S, Flores R, Di Serio F (2012) Small RNAs containing the pathogenic determinant of a chloroplast-replicating viroid guide the degradation of a host mRNA as predicted by RNA silencing. **Plant J** 70: 991–1003

Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JD (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. **Science** 312: 436–439

Navarro L, Jay F, Nomura K, He SY, Voinnet O (2008) Suppression of the microRNA pathway by bacterial effector proteins. **Science** 321: 964–967

Nowara D, Gay A, Lacomme C, Shaw J, Ridout C, Douchkov D, Hensel G, Kumlehn J, Schweizer P (2010) HIGS: Host-induced gene silencing in the obligate biotrophic fungal pathogen *Blumeria graminis*. **Plant Cell** 22: 3130–3141

Nunes CC, Dean RA (2012) Host-induced gene silencing: A tool for understanding fungal host interaction and for developing novel disease control strategies. **Mol Plant Pathol** 13: 519–529

Nuthikattu S, McCue AD, Panda K, Fultz D, DeFraia C, Thomas EN, Slotkin RK (2013) The initiation of epigenetic silencing of active transposable elements is triggered by RDR6 and 21-22 nucleotide small interfering RNAs. **Plant Physiol** 162: 116–131

Oerke EC (2006) Crop losses to pests. **J Agr Sci** 144: 31–43

Ouyang S, Park G, Atamian HS, Han CS, Stajich JE, Kaloshian I, Borkovich KA (2014) MicroRNAs suppress NB domain genes in tomato that confer resistance to *Fusarium oxysporum*. **PLoS Pathog** 10: e1004464

Palauqui JC, Elmayan T, Pollien JM, Vaucheret H (1997) Systemic acquired silencing: Transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. **EMBO J** 16: 4738–4745

Pandey SP, Baldwin IT (2007) RNA-directed RNA polymerase 1 (RdR1) mediates the resistance of *Nicotiana attenuata* to herbivore attack in nature. **Plant J** 50: 40–53

Pandey SP, Shahi P, Gase K, Baldwin IT (2008) Herbivory-induced changes in the small-RNA transcriptome and phytohormone signaling in *Nicotiana attenuata*. **Proc Natl Acad Sci USA** 105: 4559–4564

Panwar V, McCallum B, Bakkeren G (2013) Endogenous silencing of *Puccinia triticina* pathogenicity genes through in *planta*-expressed sequences leads to the suppression of rust diseases on wheat. **Plant J** 73: 521–532

Parent JS, Bouteiller N, Elmayan T, Vaucheret H (2015) Respective contributions of Arabidopsis DCL2 and DCL4 to RNA silencing. **Plant J** 81: 223–232

Pikaard CS, Haag JR, Pontes OM, Blevins T, Cocklin R (2012) A transcription fork model for Pol IV and Pol V-dependent RNA-directed DNA methylation. **Cold Spring Harb Symp Quant Biol** 77: 205–212

Pinweha N, Asvarak T, Viboonjun U, Narangajavana J (2015) Involvement of *miR160/miR393* and their targets in cassava responses to anthracnose disease. **J Plant Physiol** 174: 26–35

Pitino M, Coleman AD, Maffei ME, Ridout CJ, Hogenhout SA (2011) Silencing of aphid genes by dsRNA feeding from plants. **PLoS ONE** 6: e25709

Pradhan B, Naqvi AR, Saraf S, Mukherjee SK, Dey N (2015) Prediction and characterization of Tomato leaf curl New Delhi virus (ToLCNDV) responsive novel microRNAs in *Solanum lycopersicum*. **Virus Res** 195: 183–195

Prince DC, Drurey C, Zipfel C, Hogenhout S (2014) The leucine-rich repeat receptor-like kinase BAK1 and the cytochrome P450 PAD3 contribute to innate immunity to aphids in Arabidopsis. **Plant Physiol**: pp. 114.235598

Qazi J, Amin I, Mansoor S, Iqbal MJ, Briddon RW (2007) Contribution of the satellite encoded gene βC1 to cotton leaf curl disease symptoms. **Virus Res** 128: 135–139

Qi X, Bao FS, Xie Z (2009) Small RNA deep sequencing reveals role for *Arabidopsis thaliana* RNA-dependent RNA polymerases in viral siRNA biogenesis. **PLoS ONE** 4: e4971

Qiao Y, Liu L, Xiong Q, Flores C, Wong J, Shi J, Wang X, Liu X, Xiang Q, Jiang S, Zhang F, Wang Y, Judelson HS, Chen X, Ma W (2013) Oomycete pathogens encode RNA silencing suppressors. **Nat Genet** 45: 330–333

Qiao Y, Shi J, Zhai Y, Hou Y, Ma W (2015) Phytophthora effector targets a novel component of small RNA pathway in plants to promote infection. **Proc Natl Acad Sci USA** 112: 5850–5855

Qu J, Ye J, Fang R (2007) Artificial microRNA-mediated virus resistance in plants. **J Virol** 81: 6690–6699

Raja P, Jackel JN, Li S, Heard IM, Bisaro DM (2014) *Arabidopsis* double-stranded RNA binding protein DRB3 participates in methylation-mediated defense against geminiviruses. **J Virol** 88: 2611–2622

Raja P, Sanville BC, Buchmann RC, Bisaro DM (2008) Viral genome methylation as an epigenetic defense against geminiviruses. **J Virol** 82: 8997–9007

Rasmann S, De Vos M, Casteel CL, Tian D, Halitschke R, Sun JY, Agrawal AA, Felton GW, Jander G (2012) Herbivory in the previous generation primes plants for enhanced insect resistance. **Plant Physiol** 158: 854–863

Robert-Seilaniantz A, MacLean D, Jikumaru Y, Hill L, Yamaguchi S, Kamiya Y, Jones JD (2011) The microRNA miR393 re-directs secondary metabolite biosynthesis away from camalexin and towards glucosinolates. **Plant J** 67: 218–231

Rogers K, Chen X (2013) Biogenesis, turnover, and mode of action of plant microRNAs. **Plant Cell** 25: 2383–2399

Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM (1998) The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. **Proc Natl Acad Sci USA** 95: 9750–9754

Sanford JC, Johnston SA (1985) The concept of parasite-derived resistance - Deriving resistance genes from the parasites own genome. **J Theor Biol** 113: 395–405

Sarkies P, Miska EA (2014) Small RNAs break out: The molecular cell biology of mobile small RNAs. **Nat Rev Mol Cell Biol** 15: 525–535

Saze H, Kakutani T (2007) Heritable epigenetic mutation of a transposon-flanked *Arabidopsis* gene due to lack of the chromatin-remodeling factor DDM1. **EMBO J** 26: 3641–3652

Schwach F, Vaistij FE, Jones L, Baulcombe DC (2005) An RNA-dependent RNA polymerase prevents meristem invasion by potato virus X and is required for the activity but not the production of a systemic silencing signal. **Plant Physiol** 138: 1842–1852

Scoville AG, Barnett LL, Bodbyl-Roels S, Kelly JK, Hileman LC (2011) Differential regulation of a MYB transcription factor is correlated with transgenerational epigenetic inheritance of trichome density in *Mimulus guttatus*. **New Phytol** 191: 251–263

Shih JD, Hunter CP (2011) SID-1 is a dsRNA-selective dsRNA-gated channel. **RNA** 17: 1057–1065

Shimizu T, Nakano T, Takamizawa D, Desaki Y, Ishii-Minami N, Nishizawa Y, Minami E, Okada K, Yamane H, Kaku H, Shibuya N (2010) Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. **Plant J** 64: 204–214

Shimura H, Pantaleo V, Ishihara T, Myojo N, Inaba J, Sueda K, Burgyan J, Masuta C (2011) A viral satellite RNA induces yellow symptoms on tobacco by targeting a gene involved in chlorophyll biosynthesis using the RNA silencing machinery. **PLoS Pathog** 7: e1002021

Shivaprasad PV, Chen HM, Patel K, Bond DM, Santos BA, Baulcombe DC (2012) A microRNA superfamily regulates nucleotide binding site-leucine-rich repeats and other mRNAs. **Plant Cell** 24: 859–874

Slaughter A, Daniel X, Flors V, Luna E, Hohn B, Mauch-Mani B (2012) Descendants of primed Arabidopsis plants exhibit resistance to biotic stress. **Plant Physiol** 158: 835–843

Smith CM, Boyko EV (2007) The molecular bases of plant resistance and defense responses to aphid feeding: Current status. **Entomol Exp Appl** 122: 1–16

Smith NA, Eamens AL, Wang MB (2011) Viral small interfering RNAs target host genes to mediate disease symptoms in plants. **PLoS Pathog** 7: e1002022

Soosaar JL, Burch-Smith TM, Dinesh-Kumar SP (2005) Mechanisms of plant resistance to viruses. **Nat Rev Microbiol** 3: 789–798

Sun Z, He Y, Li J, Wang X, Chen J (2015) Genome-wide characterization of rice black streaked dwarf virus-responsive microRNAs in rice leaves and roots by small RNA and degradome sequencing. **Plant Cell Physiol** 56: 688–699

Tao XY, Xue XY, Huang YP, Chen XY, Mao YB (2012) Gossypol-enhanced P450 gene pool contributes to cotton bollworm tolerance to a pyrethroid insecticide. **Mol Ecol** 21: 4371–4385

Tsuda K, Katagiri F (2010) Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity. **Curr Opin Plant Biol** 13: 459–465

Vaucheret H (2006) Post-transcriptional small RNA pathways in plants: Mechanisms and regulations. **Genes Dev** 20: 759–771

Vaughn T, Cavato T, Brar G, Coombe T, DeGooyer T, Ford S, Groth M, Howe A, Johnson S, Kolacz K (2005) A method of controlling corn rootworm feeding using a protein expressed in transgenic maize. **Crop Sci** 45: 931–938

Wang XB, Jovel J, Udomporn P, Wang Y, Wu QF, Li WX, Gasciolli V, Vaucheret H, Ding SW (2011) The 21-nucleotide, but not 22-

nucleotide, viral secondary small interfering RNAs direct potent antiviral defense by two cooperative Argonautes in *Arabidopsis thaliana*. **Plant Cell** 23: 1625–1638

Wang XB, Wu QF, Ito T, Cillo F, Li WX, Chen XM, Yu JL, Ding SW (2010) RNAi-mediated viral immunity requires amplification of virus-derived siRNAs in *Arabidopsis thaliana*. **Proc Natl Acad Sci USA** 107: 484–489

Weiberg A, Bellinger M, Jin H (2015) Conversations between kingdoms: Small RNAs. **Curr Opin Biotechnol** 32: 207–215

Weiberg A, Wang M, Bellinger M, Jin H (2014) Small RNAs: A new paradigm in plant-microbe interactions. **Annu Rev Phytopathol** 52: 495–516

Weiberg A, Wang M, Lin FM, Zhao H, Zhang Z, Kaloshian I, Huang HD, Jin H (2013) Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. **Science** 342: 118–123

Wingard SA (1928) Hosts and symptoms of ring spot, a virus disease of plants. **J Agr Res** 37: 0127–0153

Wroblewski T, Piskurewicz U, Tomczak A, Ochoa O, Michelmore RW (2007) Silencing of the major family of NBS-LRR-encoding genes in lettuce results in the loss of multiple resistance specificities. **Plant J** 51: 803–818

Wu J, Yang Z, Wang Y, Zheng L, Ye R, Ji Y, Zhao S, Ji S, Liu R, Xu L, Zheng H, Zhou Y, Zhang X, Cao X, Xie L, Wu Z, Qi Y, Li Y (2015) Viral-inducible Argonaute18 confers broad-spectrum virus resistance in rice by sequestering a host microRNA. **Elife** 4: e05733

Wu L, Zhou H, Zhang Q, Zhang J, Ni F, Liu C, Qi Y (2010) DNA methylation mediated by a microRNA pathway. **Mol Cell** 38: 465–475

Xia K, Wang R, Ou X, Fang Z, Tian C, Duan J, Wang Y, Zhang M (2012) *OsTIR1* and *OsAFB2* downregulation via *OsmiR393* overexpression leads to more tillers, early flowering and less tolerance to salt and drought in rice. **PLoS ONE** 7: e30039

Xu L, Duan X, Lv Y, Zhang X, Nie Z, Xie C, Ni Z, Liang R (2014) Silencing of an aphid carboxylesterase gene by use of plant-mediated RNAi impairs *Sitobion avenae* tolerance of Phoxim insecticides. **Transgenic Res** 23: 389–396

Yang L, Huang H (2014) Roles of small RNAs in plant disease resistance. **J Integr Plant Biol** 56: 962–970

Yang X, Xie Y, Raja P, Li S, Wolf JN, Shen Q, Bisaro DM, Zhou X (2011) Suppression of methylation-mediated transcriptional gene silencing by βC1-SAHH protein interaction during geminivirus-betasatellite infection. **PLoS Pathog** 7: e1002329

Yi H, Richards EJ (2007) A cluster of disease resistance genes in *Arabidopsis* is coordinately regulated by transcriptional activation and RNA silencing. **Plant Cell** 19: 2929–2939

Ying XB, Dong L, Zhu H, Duan CG, Du QS, Lv DQ, Fang YY, Garcia JA, Fang RX, Guo HS (2010) RNA-dependent RNA polymerase 1 from *Nicotiana tabacum* suppresses RNA silencing and enhances viral infection in *Nicotiana benthamiana*. **Plant Cell** 22: 1358–1372

Zamore PD, Haley B (2005) Ribo-gnome: The big world of small RNAs. **Science** 309: 1519–1524

Zhai JX, Jeong DH, De Paoli E, Park S, Rosen BD, Li YP, Gonzalez AJ, Yan Z, Kitto SL, Grusak MA, Jackson SA, Stacey G, Cook DR, Green PJ, Sherrier DJ, Meyers BC (2011) MicroRNAs as master regulators of the plant *NB-LRR* defense gene family via the production of phased, *trans*-acting siRNAs. **Genes Dev** 25: 2540–2553

Zhang J, Khan SA, Hasse C, Ruf S, Heckel DG, Bock R (2015) Pest control. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. **Science** 347: 991–994

Zhang W, Gao S, Zhou X, Chellappan P, Chen Z, Zhang X, Fromuth N, Coutino G, Coffey M, Jin H (2011a) Bacteria-responsive microRNAs regulate plant innate immunity by modulating plant hormone networks. **Plant Mol Biol** 75: 93–105

Zhang XM, Zhao HW, Gao S, Wang WC, Katiyar-Agarwal S, Huang HD, Raikhel N, Jin HL (2011b) *Arabidopsis* argonaute 2 regulates innate immunity via miRNA393*-mediated silencing of a golgi-localized SNARE gene, *MEMB12*. **Mol Cell** 42: 356–366

Zhao H, Sun R, Albrecht U, Padmanabhan C, Wang A, Coffey MD, Girke T, Wang Z, Close TJ, Roose M, Yokomi RK, Folimonova S, Vidalakis G, Rouse R, Bowman KD, Jin H (2013) Small RNA profiling reveals phosphorus deficiency as a contributing factor in symptom expression for citrus huanglongbing disease. **Mol Plant** 6: 301–310

Zhong SH, Liu JZ, Jin H, Lin L, Li Q, Chen Y, Yuan YX, Wang ZY, Huang H, Qi YJ, Chen XY, Vaucheret H, Chory J, Li J, He ZH (2013) Warm temperatures induce transgenerational epigenetic release of RNA silencing by inhibiting siRNA biogenesis in *Arabidopsis*. **Proc Natl Acad Sci USA** 110: 9171–9176

Zhu QH, Fan L, Liu Y, Xu H, Llewellyn D, Wilson I (2013) miR482 regulation of NBS-LRR defense genes during fungal pathogen infection in cotton. **PLoS ONE** 8: e84390

Zhuo Y, Gao G, Shi JA, Zhou X, Wang X (2013) miRNAs: Biogenesis, origin and evolution, functions on virus-host interaction. **Cell Physiol Biochem** 32: 499–510

Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T, Felix G (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. **Cell** 125: 749–760

Zvereva AS, Pooggin MM (2012) Silencing and innate immunity in plant defense against viral and non-viral pathogens. **Viruses** 4: 2578–2597