Roles of small RNAs in the establishment of tolerant interaction between plants and viruses

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Abstract

In a tolerant plant-virus interaction, viral multiplication is sustained without substantial effects on plant growth or reproduction. Such interactions are, in natural environments, frequent and sometimes even beneficial for both interactors. Here we compiled evidence showing that small RNAs modulate plant immune responses and growth, hence adjusting its physiology to enable a tolerant interaction. Importantly, the role of small RNAs in tolerant interactions resembles that required for establishment of a mutualistic symbiosis. Tolerance can become a sustainable strategy for breeding for virus resistance as selection pressure for emergence of more aggressive strains is low. Understanding the processes underlying establishment of tolerance is therefore important for the development of future crops.

Introduction: Tolerance in-between disease and resistance

Plant interactions with viruses rely on interplay of different processes; processes supporting viral amplification, plant defence responses and viral counter-defence mechanisms. The balance between these defines the outcome of the interaction, resulting in either resistance (no viral multiplication) or disease (virus spreads within the plant causing dysfunction of plant metabolism). Studies of virus-plant interactions in natural environments, however, showed that plants and viruses often coexist [1]. Although a significant virus load is supported, plant growth, yield and reproduction are only minimally affected and visible symptoms are either absent or mild. This type of interaction between plant and virus is termed tolerance. Tolerance can be explained as reaching an equilibrium between different defensive and

pathogenic processes to allow mutually advantageous compromises in host and virus fitness for long-term coexistence [2].

Plant defence against virus infections is multilayered and includes RNA silencing, pattern-triggered (PTI)- and effector-triggered (ETI)-immunity. Following virus entry into the plant cell, certain virus-derived molecules are perceived as microbe-associated molecular patterns (MAMPs) by plant pattern recognition receptors (PRRs) and induce PTI [3–5]. Some viruses encode specialized effector proteins that are able to suppress this defence layer to promote their virulence [6]. In turn, many plants have evolved resistance (*R*) genes which usually code for nucleotide-binding site-leucine-rich repeat (NBS-LRR) receptor proteins that mediate intracellular specific recognition of viral effector proteins and initiate ETI [6,7]. RNA silencing is regarded as an adaptive form of antiviral immunity [8]. Inducers of RNA silencing are virus-derived dsRNAs, which are recognized by DICER-like (DCL) proteins and then processed into virus-derived small interfering siRNAs (vsiRNAs). vsiRNAs are recruited into different Argonaute (AGO) protein(s) forming RNA-induced silencing complex (RISC) that act against viral RNA molecules. Cleaved viral RNA further serves as a template for host RNA-dependent RNA polymerase (RDR) to synthesize de novo dsRNAs thus boosting antiviral silencing effect [9]. Many viruses however adaptively evolved to evade this type of immunity by encoding viral suppressors of RNA silencing (VSRs) as viral effectors that interfere with nearly every step of the RNA silencing pathway [10–12].

RNA silencing also has an important function in regulation of plant endogenous processes (reviewed in [11]. Plants synthesize different small RNAs (sRNAs), classified into microRNAs (miRNAs) and small interfering RNAs (siRNAs) on the basis of their biogenesis and structures of their precursors. Both miRNAs and siRNAs bind to AGOs to mediate post-transcriptional gene silencing (PTGS) via sRNA-directed mRNA cleavage or translational repression, or transcriptional gene silencing (TGS) via sRNA-directed DNA methylation [13,14]. To date, a plethora of plant sRNAs was found to be involved in PTI and ETI [15], showing that RNA silencing, PTI and ETI are closely linked. Components of PTI/ETI can transcriptionally regulate activity of sRNAs and sRNAs regulate activity of PTI/ETI components posttranscriptionally. Functional characterization studies of sRNA´s role in plant immune responses were mainly performed in plant-bacteria or plant-fungi pathosystems [16], while knowledge of the relationships between sRNAs and immune responses against viruses is still scarce. There is a vast number of reports describing how viral infection perturbs the endogenous sRNA levels, but many of these, so-called virus-responsive sRNAs, are yet to be functionally characterized [14].

While molecular mechanisms in resistance against viruses were intensively studied [17–19], the molecular basis of tolerance is much less understood. Disease can be the consequence of ineffective runaway immune response, metabolism shifts and cell rearrangements caused by viral multiplication or viral toxic

effects (reviewed in [20]). Thus modifying any of these can result in tolerance. On the other hand, tolerance can also be a result of active recognition of the virus by receptor kinases, tuning the responses towards the favourable outcome for both organisms.

In this review, we will succinctly summarize current knowledge of sRNAs' functions governing tolerance. We also present the available knowledge on the roles of sRNAs in disease recovery, a tolerant state plants may acquire at later stages of certain plant-virus interactions. We additionally compare the involvement of sRNAs in establishment of tolerance and mutualistic symbiosis, as we argue that tolerance can be regarded as an intermediate state in symbiotic the continuum between antagonistic and mutualistic relationships.

sRNAs at a crossroad: disease or tolerance

Interestingly, many sRNAs found to date associated with the tolerance are closely interconnected either ETI or PTI immune signalling. Alternatively, tolerance promoting sRNAs are balancing trade-offs between growth and immunity.

In rice, miR444 promotes tolerance to rice stripe virus (RSV) infection. miR444 reduces accumulation of its targets, transcripts encoding MIKCC-type MADS box proteins, *MADS23*, *MADS27*, and *MADS5*, which are transcriptional repressors of the *RDR1* gene (Figure 1) thus boosting RNA-silencing against the virus. Over-expression of miR444 resulted in milder symptoms and reduced accumulation of RSV [21]. Another monocot-specific miRNA, miR528, is involved in tolerant interaction of rice to RSV. In contrast to miR444, miR528 seems to act as a negative regulator by cleaving *L-ascorbate oxidase* (*AO*) mRNAs, thereby reducing AO-mediated accumulation of reactive oxygen species (ROS). ROS is an important signalling component in antiviral immunity [22]. *mir528* mutant plants displayed milder symptoms and accumulated less virus, whereas miR528 overexpression lines were more susceptible to RSV infection [23]. Similarly, in interaction with rice black-streaked dwarf virus (RBSDV), increased accumulation of miR528 contributed to much more severe disease symptoms, higher disease incidence and increased levels of viral RNA, whereas miR528 deficiency enhanced antiviral defence against RBSDV infection [23]. miR528 was found to be negatively regulated by the ROS, hydrogen peroxide [24], indicating that redox homeostasis is important in promoting tolerance. In diseased maize, miR528 was up-regulated after sugarcane mosaic virus infection, while miR444 was downregulated, further supporting their role as positive and negative regulators of antiviral immunity in monocots [25]. The *MIR528* gene is transcriptionally activated by the SQUAMOSA Promoter-Binding-Like 9 (SPL9) transcription factor. Similarly, as the increased level of miR528, *SPL9* overexpression leads to severe disease symptoms, higher disease incidence and increased virus accumulation [26]. Moreover, SPL9 is targeted by miR156,

which when downregulated, has been linked to disease symptom occurrence in rice and maize plants in response to RSV and RBSDV (Figure 1) [27,28].

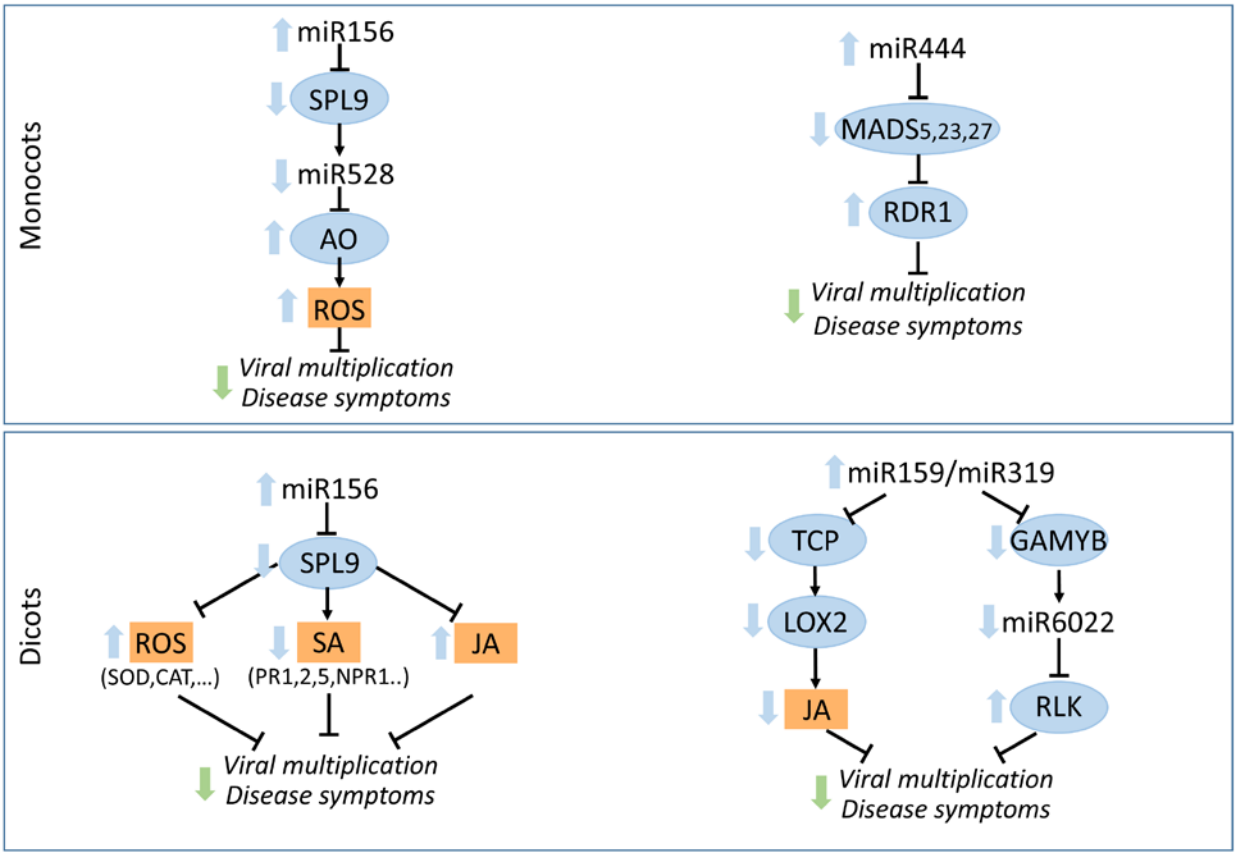

Figure 1: Role of sRNAs in plant tolerance to viral infection. Regulatory sRNA/transcript modules identified in monocots (upper panel) and in dicots (lower panel) are shown. Thick arrows indicate increase/decrease in abundance of signalling components (blue arrows) and symptoms development (green arrows), black thin arrows denote activation and bar-headed lines denote inhibition. SPL9 - SQUAMOSA Promoter-Binding-Like 9 transcription factor, AO - L-ascorbate oxidase, ROS - reactive oxygen species, MADS - MIKCC-type MADS box protein, RDR - RNA-dependent RNA polymerase, SA - salicylic acid, JA - jasmonic acid, TCP - Teosinte branched1/cycloidea/proliferating bHLH transcription factor, GAMYB – MYB transcription factor controlling gibberellin signalling, LOX2 - lipoxygenase 2, RLK - receptor-like kinases.

In dicots, however, miR156 seem to function as a negative effector of immunity. Increased levels of miR156 were found to correlate with severity of disease symptoms in *Nicotiana benthamiana* in response to potyviruses potato virus Y (PVY) and plum pox virus, and potexvirus potato virus X [29]. Similarly, miR156 levels were significantly increased by tobacco mosaic virus, and its levels correlated with symptom severity in tobacco (*Nicotiana tabacum*) [30]. miR156 levels were also upregulated in tobacco plants infected with PVY [31]. Recently, miR156/SPLs regulatory module was found to control innate

immunity by regulating ROS accumulation and activating the salicylic acid (SA) signalling pathway in Arabidopsis. *mir156* suppression mutants or *SPL9* overexpression mutants exhibited increased ROS levels and decreased expression of SA signalling genes [32]. When challenged with virulent *Pseudomonas syringae* pv. tomato DC3000strain the same plants showed less severe symptoms and lower bacterial proliferation. SA- and ROS-signalling pathways were crucial also in antiviral immunity [22,33]. Since SPL9 also negatively regulates jasmonic acid (JA) response [34] the miR156/SPL9 module must be important in controlling tolerance responses by regulating immune signalling networks through multiple connections in dicots. Even though the module has not been yet functionally characterized in response to viral infections, the elevated levels of miR156 often detected in diseased dicot plants imply that the module might function similarly as in response to bacteria [30,31,35].

Furthermore, sRNAs link immune signalling with growth. In response to PVY infection, tolerant potato plants exhibited increased levels of miR167 and a secondary siRNA (phasiRNA931) that target transcripts encoding two gibberellin (GA) biosynthesis genes, and miR319 (closely related to Arabidopsis miR159), which targets *MYB33*, which encodes a GAMYB transcription factor involved in GA signal transduction [36]. In Arabidopsis, a GAMYB-targeting miR159 was reported to be involved in limiting disease symptoms in response to a severe strain of CMV. Derepression of miR159 targets *MYB33* and *MYB65* resulted in exacerbated disease symptoms, whereas *myb33/myb65* double knockout resulted in ameliorated symptoms [37]. Similarly, a diminished level of miR159 was detected in *N. tabacum* leaves displaying disease symptoms after CMV infection [38], further confirming the positive role of miR159/miR319 in the antiviral response. Additionally, there is a link between GA signalling and *R* gene expression, supporting the hypothesis that viral perception is actively modulated in tolerant interactions. In the potato-PVY interaction, downregulation of miR6022 (targeting *LRR-RLKs*) occurs, which is linked to downregulation of GA signalling as GAMYB binding sites were discovered in the *MIR6022* promoter region [36]. The miR159/miR319 family also targets *Teosinte branched1/cycloidea/proliferating* (*TCP)* bHLH transcription factor which targets *lipoxygenase 2* (*LOX2)* involved in JA biosynthesis [39] (Figure 1).

In monocots, by contrast, miR319 negatively regulates tolerance responses of rice to rice ragged stunt virus and of wheat to RBSDV. miR319 overexpression facilitates infection and symptom development, whereas blocking its activity results in milder symptoms and lower virus levels [39]. Similar to miR156 and miR319, miR166 is another conserved miRNA that displays a contrasting regulatory role in antiviral responses in monocot and dicot species. In dicots, suppression of miR166 expression attenuates symptom development [30,40,41]. On the other hand, decreased miR166 levels were detected in symptomatic virus-infected rice and maize [25,42].

Symbiosis and tolerance: are they more similar than we think?

Rhizobial and plant arbuscular mycorrhizal (AM) symbioses represent two of the most researched mutualistic interactions, leading to development of nitrogen-fixing nodules and mycorrhizal arbuscules, respectively [43]. Recent evidence suggests that a plethora of plant sRNAs are involved in both types of symbiosis [44,45]. One set of regulated sRNAs is associated with negative control of *R* genes (i.e. *NBS-LRRs*), which is in line with the hypothesis that balancing of plant immune responses is required to tolerate invasion and proliferation of beneficial microorganisms. Decreased disease resistance gene expression and increased levels of legume-specific miRNAs targeting them (e.g. miR5213, miR5281) were observed during AM symbiosis in *Medicago truncatula* [46]. Moreover, miR482, which targets *NBS-LRR* transcripts was reported to be induced during establishment of symbiosis between soybean and *Bradyrhizobium japonicum* [47]. Notably, miR482 which targets *NBS-LRR* transcripts was upregulated following PVY infection in the tolerant potato-PVY interaction [36]. Similarity in regulation of certain sRNAs should not be so unexpected because in both, the tolerant response to viruses and mutualistic interactions, plants need to tolerate the presence of microorganisms (Figure 2).

Many miRNAs, reported to regulate rhizobial and AM symbioses, are implicated in the direct or indirect regulation of auxin signalling genes[46,48]. This is not surprising since auxin levels are crucial for proper legume nodule and arbuscule development [49,50]. Interestingly, many miRNAs, namely miR160, miR164, miR167, miR390, miR393 were found to be similarly regulated during tolerance response of potato to PVY [36]. Also, decreased GA levels were shown to be regulated by a miRNA/phasiRNA circuit in the potato tolerance response [36] and shown to be involved in rhizobial and mycorrhizal signalling network [51–53]. This is yet another similarity between the response of plants in mutualistic symbiosis and in the tolerance response (Figure 2). Several other miRNAs that were upregulated in tolerant response to PVY in potato, such as miR169, miR171 and miR319, also regulate nodulation and AM symbiosis in different legume species [46,54]. miR171 was linked to tolerance in several viral pathosystems [30,55–57]. Rice overexpressing miR171 is less susceptible to RSV and shows attenuated disease symptoms, whereas reducing miR171 accumulation leads to development of disease symptoms [57].

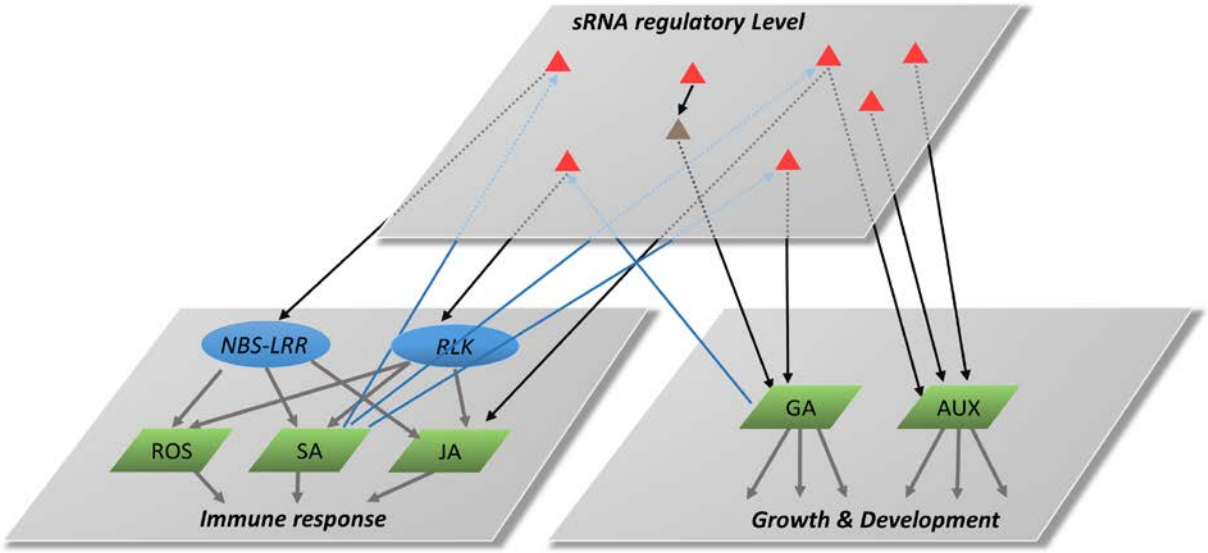

Figure 2: sRNA regulation links immunity and developmental processes in tolerance similarly as in establishment of mutualistic symbiosis. All examples of processes regulated by sRNAs in both types of interaction currently known are presented. Both, immune response and growth/developmental hormonal networks are regulated by sRNAs in both types of interaction. All regulatory levels are however tightly intertwined. miRNAs are represented by red triangles, phasiRNA is represented by a brown triangle. Arrows present relationship between sRNAs and receptors (blue ovals) or signalling modules (green rectangles), black for the regulation of transcripts by sRNA and blue for the regulation of sRNA by transcription factor in one of the modules. NBS-LRR - nucleotide-binding site-leucine-rich repeat proteins, RLK – receptor-like kinases, ROS reactive oxygen species, SA – salicylic acid, JA – jasmonic acid, – AUX – auxin, GA – gibberellin.

sRNA and disease recovery: it is never too late

Some virus-infected plants are able to recover from the disease at later stages of infection. This outcome is known as symptom recovery and is characterized by emergence of asymptomatic leaves following a systemic symptomatic infection, despite persistence of the virus [58–60]. The plant recovery phenotype thus mimics the tolerant phenotype, and disease recovery can be therefore regarded as an inducible form of tolerance [20]. One of the characteristics of recovery is that recovered leaves exhibit resistance to reinfection by a related but not unrelated viruses, suggesting that recovery is governed by a sequence-specific defence mechanism such as RNA silencing [9]. Indeed, recovery depends on AGO1 [61], the core component of antiviral silencing, on DCL4/RDR6/SGS3 PTGS pathway and on two TGS components, RDR2 and RNA polymerase IV, which are involved in the maintenance and spread of silencing [62]. Based on that, Kørner et al., proposed a model for disease recovery suggesting, that the dose of so-called

antiviral siRNAs in tissues undergoing recovery needs to surpass a critical threshold to saturate the VSR and block its activity, ultimately allowing the recovery [62]. Antiviral siRNA are thought to primarily originate from symptomatic tissues, as they contain high amounts of viral RNA available for degradation. Accordingly, removal of symptomatic leaves was shown to delay recovery [60]. Since recovery correlated with increased vsiRNA level in recovered leaves, it was suggested that vsiRNAs are the crucial molecules causing the VSR saturation [62–64]. However, the putative involvement of plant secondary siRNAs which are also produced by DCL4/RDR6/SGS3 pathway [65] was not considered. Recent sRNA-ome analysis of PVY-infected tomato before and after symptom recovery identified many differentially regulated endogenous miRNAs and secondary siRNAs, suggesting that besides vsiRNAs, plant sRNAs seem to be important for the transition from diseased to recovered conditions as well [59]. In the case of DNA viruses, such as geminiviruses, plants additionally employ a TGS pathway [66,67]. sRNA-mediated methylation of viral genome function in restriction of the transcription and movement of the virus and also promotes a recovery process [68,69].

Conclusions

For breeders, resistance is traditionally preferred over tolerance, as tolerant crops represent a virus reservoir, which might affect the sensitive varieties [20]. It was however reported that under some abiotic or biotic stress condition the latent viral infection can be even beneficial for the plant, enhancing the fitness of the host and offer protection against a larger spectrum of isolates compared to resistance [70]. Moreover, tolerance may also have an advantage over resistance for crop protection because it does not actively prevent virus infection and/or replication, therefore the selection pressure for the emergence of more aggressive strains is reduced, and it is thus likely to be more evolutionary stable than resistance [20]. sRNAs are known to modulate both immune response as well as growth and developmental signalling [36,71]. Increasing evidence shows that sRNAs govern the establishment of mutualistic symbiosis as well as tolerance, thus representing interesting markers for breeding. sRNAs, however, seem to act in complex ways, individually only contributing to a subtle effect, but by acting through multiple action points they gain their regulatory power. It seems that sRNAs are engaged with transcription factors and hormone pathways into a large network that coregulates the trade-offs between growth and immunity [72] (Figure 2). We contend that understanding this multilayered response is thus crucial for design of agriculturally efficient crops in the future.

Declarations of interest

The authors declare no conflict of interest.

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References

1. Roossinck MJ, Bazán ER: **Symbiosis: Viruses as Intimate Partners**. *Annu Rev Virol* 2017, **4**:123–139.

2. Bengyella L, Waikhom SD, Allie F, Rey C: **Virus tolerance and recovery from viral induced-symptoms in plants are associated with transcriptome reprograming**. *Plant Mol Biol* 2015, **89**:243–252.

3. Niehl A, Wyrsch I, Boller T, Heinlein M: **Double-stranded RNAs induce a pattern-triggered immune signaling pathway in plants**. *New Phytol* 2016, **211**:1008–1019.

4. Saijo Y, Loo EP, Yasuda S: **Pattern recognition receptors and signaling in plant–microbe interactions**. *Plant J* 2018, **93**:592–613.

5. Niehl A, Heinlein M: **Perception of double-stranded RNA in plant antiviral immunity**. *Mol Plant Pathol* 2019, **20**:1203–1210.

6. Gouveia BC, Calil IP, Machado JPB, Santos AA, Fontes EPB: **Immune receptors and co-receptors in antiviral innate immunity in plants**. *Front Microbiol* 2017, **7**:1–14.

7. Dangl JL, Jones JDG: **A pentangular plant inflammasome**. *Science* 2019, **364**:31–32.

8. Carr JP, Murphy AM, Tungadi T, Yoon JY: **Plant defense signals: Players and pawns in plant-virus-vector interactions**. *Plant Sci* 2018, **278**:87–95.

** This work comprehensively summarises the multilayed nature of plant signaling in plant-virus-vector interactions

9. Baulcombe D: **RNA silencing in plants.** *Nature* 2004, **431**:356–363.

10. Yang Z, Li Y: **Dissection of RNAi-based antiviral immunity in plants**. *Curr Opin Virol* 2018, **32**:88–99.

11. Huang CY, Wang H, Hu P, Hamby R, Jin H: **Small RNAs – Big Players in Plant-Microbe Interactions**. *Cell Host Microbe* 2019, **26**:173–182.

* In this review the authors compile information on endogenous function of sRNAs and RNAi machinery in plant-microbe interactions and highlight the recent discoveries of cross-kingdom RNAi

12. Gaffar FY, Koch A: **Catch Me If You Can! RNA Silencing-Based Improvement of Antiviral**

Plant Immunity. *Viruses* 2019, **11**:1–32.

* In this paper, the authors review the recent studies which reveal the enormous potential that RNA-silencing strategies hold for providing an environmentally friendly mechanism to protect crop plants from viral diseases

13. Borges F, Martienssen RA: **The expanding world of small RNAs in plants**. *Nat Publ Gr* 2015, **16**:1–15.

14. Zhang B, Li W, Zhang J, Wang L, Wu J: **Roles of Small RNAs in Virus-Plant Interactions**. *Viruses* 2019, **11**:1–16.

15. Fei Q, Zhang Y, Xia R, Meyers BC: **Small RNAs Add Zing to the Zig-Zag-Zig Model of Plant Defenses**. *Mol Plant-Microbe Interact* 2016, **29**:165–169.

16. Song X, Li Y, Cao X, Qi Y: **MicroRNAs and Their Regulatory Roles in Plant–Environment Interactions**. *Annu Rev Plant Biol* 2019, **70**:489–525.

17. de Ronde D, Butterbach P, Kormelink R: **Dominant resistance against plant viruses.** *Front Plant Sci* 2014, **5**:1–17.

18. Sanfaçon H: **Plant translation factors and virus resistance**. *Viruses* 2015, **7**:3392–3419.

19. Hashimoto M, Neriya Y, Yamaji Y, Namba S: **Recessive resistance to plant viruses: Potential resistance genes beyond translation initiation factors**. *Front Microbiol* 2016, **7**:1–11.

20. Paudel DB, Sanfaçon H: **Exploring the Diversity of Mechanisms Associated With Plant Tolerance to Virus Infection**. *Front Plant Sci* 2018, **9**:1–20.

** In this review the authors summarise processes that plants employ to allow tolerance to virus infection.

21. Wang H, Jiao X, Kong X, Hamera S, Wu Y, Chen X, Fang R, Yan Y: **A signaling cascade from miR444 to RDR1 in rice antiviral RNA silencing pathway**. *Plant Physiol* 2016, **170**:2365–2377.

22. Mandadi KK, Scholthof K-BG: **Plant Immune Responses Against Viruses: How Does a Virus Cause Disease?** *Plant Cell* 2013, **25**:1489–1505.

23. Wu J, Yang R, Yang Z, Yao S, Zhao S, Wang Y, Li P, Song X, Jin L, Zhou T, et al.: **ROS accumulation and antiviral defence control by microRNA528 in rice**. *Nat Plants* 2017, **3**:1–7.

24. Li T, Li H, Zhang YX, Liu JY: **Identification and analysis of seven H2O2-responsive miRNAs and 32 new miRNAs in the seedlings of rice (Oryza sativa L. ssp. indica)**. *Nucleic Acids Res* 2011, **39**:2821–2833.

25. Xia Z, Zhao Z, Li M, Chen L, Jiao Z, Wu Y, Zhou T, Yu W, Fan Z: **Identification of miRNAs and their targets in maize in response to Sugarcane mosaic virus infection**. *Plant Physiol Biochem* 2018, **125**:143–152.

26. Yao S, Yang Z, Yang R, Huang Y, Guo G, Kong X, Lan Y, Zhou T, Wang H, Wang W, et al.: **Transcriptional Regulation of miR528 by OsSPL9 Orchestrates Antiviral Response in Rice**. *Mol Plant* 2019, **12**:1114–1122.

* In this paper the authors decribe a new regulatory layer of miR528-ROS signaling defence pathway.

27. Zhou Y, Xu Z, Duan C, Chen Y, Meng Q, Wu J, Hao Z, Wang Z, Li M, Yong H, et al.: **Dual transcriptome analysis reveals insights into the response to Rice black-streaked dwarf virus in maize**. *J Exp Bot* 2016, **67**:4593–4609.

28. Yang J, Zhang F, Li J, Chen JP, Zhang HM: **Integrative analysis of the microRNAome and transcriptome illuminates the response of susceptible rice plants to rice stripe virus**. *PLoS One* 2016, **11**:1–21.

29. Pacheco R, García-Marcos A, Barajas D, Martiáñez J, Tenllado F: **PVX–potyvirus synergistic infections differentially alter microRNA accumulation in Nicotiana benthamiana**. *Virus Res* 2012, **165**:231–235.

30. Bazzini a a, Hopp HE, Beachy RN, Asurmendi S: **Infection and coaccumulation of tobacco mosaic virus proteins alter microRNA levels, correlating with symptom and plant development.** *Proc Natl Acad Sci U S A* 2007, **104**:12157–12162.

31. Guo Y, Jia MA, Yang Y, Zhan L, Cheng X, Cai J, Zhang J, Yang J, Liu T, Fu Q, et al.: **Integrated analysis of tobacco miRNA and mRNA expression profiles under PVY infection provids insight into tobacco-PVY interactions**. *Sci Rep* 2017, **7**:1–10.

32. Yin H, Hong G, Li L, Zhang X, Kong Y, Sun Z, Li J, Chen J, He Y: **MiR156/SPL9 regulates reactive oxygen species accumulation and immune response in arabidopsis thaliana**. *Phytopathology* 2019, **109**:632–642.

33. Stare T, Ramšak Ž, Blejec A, Stare K, Turnšek N, Weckwerth W, Wienkoop S, Vodnik D, Gruden K: **Bimodal dynamics of primary metabolism-related responses in tolerant potato-Potato virus Y interaction.** *BMC Genomics* 2015, **16**:1–17.

34. Mao YB, Liu YQ, Chen DY, Chen FY, Fang X, Hong GJ, Wang LJ, Wang JW, Chen XY: **Jasmonate response decay and defense metabolite accumulation contributes to age-regulated dynamics of plant insect resistance**. *Nat Commun* 2017, **8**:1–13.

35. Pacheco R, García-Marcos A, Barajas D, Martiáñez J, Tenllado F: **PVX-potyvirus synergistic infections differentially alter microRNA accumulation in Nicotiana benthamiana**. *Virus Res* 2012, **165**:231–235.

36. Križnik M, Petek M, Dobnik D, Ramšak Ž, Baebler Š, Pollmann S, Kreuze J, Gruden K: **Salicylic Acid Perturbs sRNA-Gibberellin Regulatory Network in Immune Response of Potato to Potato virus Y Infection**. 2017, **8**:1–14.

37. Du Z, Chen A, Chen W, Westwood JH, Baulcombe DC, Carr JP: **Using a viral vector to reveal the role of MicroRNA159 in disease symptom induction by a severe strain of Cucumber mosaic virus**. *Plant Physiol* 2014, **164**:1378–1388.

38. Lang Q, Jin C, Lai L, Feng J, Chen S, Chen J: **Tobacco microRNAs prediction and their expression infected with Cucumber mosaic virus and Potato virus X**. *Mol Biol Rep* 2011, **38**:1523–1531.

39. Zhang C, Ding Z, Wu K, Yang L, Li Y, Yang Z, Shi S, Liu X, Zhao S, Yang Z, et al.: **Suppression of Jasmonic Acid-Mediated Defense by Viral-Inducible MicroRNA319 Facilitates Virus Infection in Rice**. *Mol Plant* 2016, **9**:1302–1314.

40. Wang S, Cui W, Wu X, Yuan Q, Zhao J, Zheng H, Lu Y, Peng J, Lin L, Chen J, et al.: **Suppression of nbe-miR166h-p5 attenuates leaf yellowing symptoms of potato virus X on Nicotiana benthamiana and reduces virus accumulation**. *Mol Plant Pathol* 2018, **19**:2384–2396.

41. Yin Z, Murawska Z, Xie F, Pawełkowicz M, Michalak K, Zhang B, Lebecka R: **microRNA response in potato virus Y infected tobacco shows strain-specificity depending on host and symptom severity**. *Virus Res* 2019, **260**:20–32.

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

43. Hussain SS, Hussain M, Irfan M, Siddique KHM: **Legume, Microbiome, and Regulatory Functions of miRNAs in Systematic Regulation of Symbiosis**. In *Plant Microbiome: Stress Response*. Edited by Egamberdieva D, Ahmad P. Springer Nature Singapore; 2018:255–282.

44. Lelandais-Briere C, Moreau J, Hartmann C, Crespi M: **Noncoding RNAs , Emerging Regulators in Root Endosymbioses**. 2016, **29**:170–180.

45. Hobecker KV, Reynoso MA, Bustos-Sanmamed P, Wen J, Mysore KS, Crespi M, Blanco FA, Zanetti ME: **The MicroRNA390/TAS3 Pathway Mediates Symbiotic Nodulation and Lateral Root Growth**. *Plant Physiol* 2017, **174**:2469–2486.

46. Devers EA, Branscheid A, May P, Krajinski F: **Stars and Symbiosis : MicroRNA- and MicroRNA * -Mediated Transcript Cleavage Involved in Arbuscular Mycorrhizal Symbiosis**. 2011, **156**:1990–2010.

47. Li H, Deng Y, Wu TL, Subramanian S, Yu O: **Misexpression of miR482, miR1512, and miR1515 increases soybean nodulation**. *Plant Physiol* 2010, **153**:1759–1770.

48. Allen E, Xie Z, Gustafson AM, Carrington JC: **microRNA-Directed Phasing during Trans-Acting siRNA Biogenesis in Plants**. *Cell* 2005, **121**:207–221.

49. Nizampatnam NR, Schreier SJ, Damodaran S, Adhikari S, Subramanian S: **microRNA160 dictates stage-specific auxin and cytokinin sensitivities and directs soybean nodule development**. *Plant J* 2015, **84**:140–153.

50. Etemadi M, Gutjahr C, Couzigou JM, Zouine M, Lauressergues D, Timmers A, Audran C, Bouzayen M, Bécard G, Combier JP: **Auxin perception is required for arbuscule development in arbuscular mycorrhizal symbiosis**. *Plant Physiol* 2014, **166**:281–292.

51. Floss DS, Levy JG, Lévesque-tremblay V, Pumplin N, Harrison MJ: **DELLA proteins regulate arbuscule formation in arbuscular mycorrhizal symbiosis**. 2013, **2013**:5025–5034.

52. Fonouni-Farde C, Tan S, Baudin M, Brault M, Wen J, Mysore KS, Niebel A, Frugier F, Diet A: **DELLA-mediated gibberellin signalling regulates Nod factor signalling and rhizobial infection**. *Nat Commun* 2016, **7**:1–13.

53. Jin Y, Liu H, Luo D, Yu N, Dong W, Wang C, Zhang X, Dai H, Yang J, Wang E: **DELLA proteins are common components of symbiotic rhizobial and mycorrhizal signalling pathways**. *Nat Commun* 2016, **7**:1–14.

54. Lelandais-Brière C, Naya L, Sallet E, Calenge F, Frugier F, Hartmann C, Gouzy J, Crespi M: **Genome-wide Medicago truncatula small RNA analysis revealed novel microRNAs and isoforms differentially regulated in roots and nodules.** *Plant Cell* 2009, **21**:2780–96.

55. Naqvi AR, Haq QMR, Mukherjee SK: **MicroRNA profiling of tomato leaf curl new delhi virus (tolcndv) infected tomato leaves indicates that deregulation of mir159/319 and mir172 might be linked with leaf curl disease**. *Virol J* 2010, **7**:1–16.

56. Feng J, Wang Y, Lin R, Chen J: **Altered Expression of MicroRNAs and Target mRNAs in Tomato Root and Stem Tissues upon Different Viral Infection**. *J Phytopathol* 2013, **161**:107–119.

57. Tong A, Yuan Q, Wang S, Peng J, Lu Y, Zheng H, Lin L, Chen H, Gong Y, Chen J, et al.: **Altered accumulation of osa-miR171b contributes to rice stripe virus infection by regulating disease symptoms**. *J Exp Bot* 2017, **68**:4357–4367.

58. Nemes K, Gellért Á, Bóka K, Vági P, Salánki K: **Symptom recovery is affected by Cucumber mosaic virus coat protein phosphorylation**. *Virology* 2019, **536**:68–77.

59. Prigigallo MI, Križnik M, Paola D De, Catalano D, Gruden K, Finetti-Sialer MM, Cillo F: **Potato Virus Y Infection Alters Small RNA Metabolism and Immune Response in Tomato**. *Viruses* 2019, **11**:1–27.

* Here authors describe the role of small RNAs in establishment of recovery to virus PVY in tomato

60. Nie X, Molen TA: **Host recovery and reduced virus level in the upper leaves after Potato virus Y infection occur in tobacco and tomato but not in potato plants**. *Viruses* 2015, **7**:680–698.

61. Ghoshal B, Sanfaçon H: **Temperature-dependent symptom recovery in Nicotiana benthamiana plants infected with tomato ringspot virus is associated with reduced translation of viral RNA2 and requires ARGONAUTE 1**. *Virology* 2014, **456**–**457**:188–197.

62. Kørner CJ, Pitzalis N, Peña EJ, Erhardt M, Vazquez F, Heinlein M: **Crosstalk between PTGS and TGS pathways in natural antiviral immunity and disease recovery**. *Nat Plants* 2018, **4**:157–164.

*** In this study the authors demonstrate that recovery depends on the siRNA-mediated post-transcriptional gene silencing pathway and on components of a transcriptional gene silencing pathway that is known to facilitate non-cell autonomous silencing signalling. They also speculate that establishment of recovery occurs folowing robust delivery of antiviral siRNAs from source to sink tissues.

63. Rodriguez-Negrete EA, Carrillo-Tripp J, Rivera-Bustamante RF: **RNA Silencing against Geminivirus: Complementary Action of Posttranscriptional Gene Silencing and Transcriptional Gene Silencing in Host Recovery**. *J Virol* 2009, **83**:1332–1340.

64. Chellappan P, Vanitharani R, Pita J, Fauquet CM: **Short Interfering RNA Accumulation Correlates with Host Recovery in DNA Virus-Infected Hosts, and Gene Silencing Targets Specific Viral Sequences**. *J Virol* 2006, **80**:1064–1064.

65. Deng P, Muhammad S, Cao M, Wu L: **Biogenesis and regulatory hierarchy of phased small interfering RNAs in plants**. *Plant Biotechnol J* 2018, **16**:965–975.

**This review discusses the findings about biogenesis, targeting modes and regulatory networks of plant phasiRNAs

66. Ramesh S V., Sahu PP, Prasad M, Praveen S, Pappu HR: **Geminiviruses and plant hosts: A closer examination of the molecular arms race**. *Viruses* 2017, **9**:1–21.

67. Raja P, Jackel JN, Li S, Heard IM, Bisaro DM: **Arabidopsis Double-Stranded RNA Binding Protein DRB3 Participates in Methylation-Mediated Defense against Geminiviruses**. *J Virol* 2013, **88**:2611–2622.

68. Wang C, Wang C, Zou J, Yang Y, Li Z, Zhu S: **Epigenetics in the plant–virus interaction**. *Plant Cell Rep* 2019, **38**:1031–1038.

69. Coursey T, Regedanz E, Bisaro DM: **Arabidopsis RNA Polymerase V Mediates Enhanced Compaction and Silencing of Geminivirus and Transposon Chromatin during Host Recovery**

from Infection. *J Virol* 2018, **92**:1–18.

70. Aguilar E, Cutrona C, del Toro FJ, Vallarino JG, Osorio S, Pérez-Bueno ML, Barón M, Chung BN, Canto T, Tenllado F: **Virulence determines beneficial trade-offs in the response of virus-infected plants to drought via induction of salicylic acid**. *Plant Cell Environ* 2017, **40**:2909–2930.

71. Deng Y, Wang J, Tung J, Liu D, Zhou Y, He S, Du Y, Baker B, Li F: **A role for small RNA in regulating innate immunity during plant growth**. 2018, **14**:1–22.

72. Ning Y, Liu W, Wang GL: **Balancing Immunity and Yield in Crop Plants**. *Trends Plant Sci* 2017, **22**:1069–1079.