Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Virology

The battle for survival between viruses and their host plants

Adnane Boualem[1], Catherine Dogimont[2] and Abdelhafid Bendahmane[1]

Evolution has equipped plants with defense mechanisms to counterattack virus infections. However, some viruses have acquired the capacity to escape these defense barriers. In their combats, plants use mechanisms such as antiviral RNA silencing that viruses fight against using silencing-repressors. Plants could also resist by mutating a host factor required by the virus to complete a particular step of its infectious cycle. Another successful mechanism of resistance is the hypersensitive response, where plants engineer R genes that recognize specifically their assailants. The recognition is followed by the triggering of a broad spectrum resistance. New understanding of such resistance mechanisms will probably helps to propose new means to enhance plant resistance against viruses.

Addresses
[1] Institute of Plant Sciences Paris-Saclay, IPS2, INRA, CNRS, Univ. Paris-Sud, Univ. d'Evry, Univ. Paris-Diderot, Sorbonne Paris-Cite, Université Paris-Saclay, Batiment 630, Rue de Noetzlin, Orsay 91405, France
[2] INRA, UR 1052, Unité de Génétique et d'Amélioration des Fruits et Légumes, BP 94, Montfavet F-84143, France

Corresponding author: Bendahmane, Abdelhafid (bendahm@evry.inra.fr)

Current Opinion in Virology 2016, **17**:32–38

This review comes from a themed issue on **Viral pathogenesis**

Edited by **Vicente Pallas** and **Juan A. García**

For a complete overview see the Issue and the Editorial

Available online 19th January 2016

http://dx.doi.org/10.1016/j.coviro.2015.12.001

Introduction

Plants are constantly challenged by pathogens from all kingdoms like nematodes, fungi, bacteria and viruses. To defend themselves and prevent disease, plants have evolved sophisticated and efficient mechanisms. One of the most common disease defense is the induction of a rapid localized cell death at the point of pathogen infection, called hypersensitive response (HR). The HR can be triggered by a wide variety of pathogens, including viruses, and relies mainly on dominant resistance (R) genes, which recognize pathogen-derived effector proteins. In this short review, we intend to first, provide a brief overview of severe virus-associated plant diseases and their impact on crop production, second, to present the current state of knowledge on vectors for virus transmission, and third, to summarize recent progress in understanding plant resistance against viruses focusing on the R genes mediated dominant resistance.

Viruses and diseases

Viruses are obligate intracellular parasites absolutely dependent on the host cell machinery to multiply and spread. They are nucleic acid-based pathogens with genomes that consist of single-stranded or double-stranded RNAs or DNAs encoding few genes and usually packed into protein envelopes called the capsid. Viruses invade all forms of life and viral infection causes physiological disorders leading to diseases. Viral diseases are undoubtedly one of the most limiting factors that cause significant yield loss and continuously threaten crop production worldwide. Damages range from stunted growth, reduced vigor, decreased market esthetic values of the products and/or total yield loss. Although it's very complex to put a clear figure on the economic impact of plant diseases in agriculture, it was estimated that 15% of global crop production is lost due to pre-harvest plant disease [1] and viruses account for 47% of the plant diseases [2]. In South-East Asia, viruses such as the tungro viral disease (*Rice tungro spherical virus* and *Rice tungro bacilliform virus*), the *Rice yellow mottle virus* (RYMV) and the *Rice stripe virus* (RSV) were reported to cause yield losses of 50–100% estimated to an annual economic loss of more than US$1.5 billion [3••]. In East and Central Africa, the *African cassava mosaic virus* (ACMV), the major constraint for cassava cultivation, was reported to cause yield losses of 47% of the production corresponding to economic loss of more than US$2 billion [4].

Virus-transmitting vectors

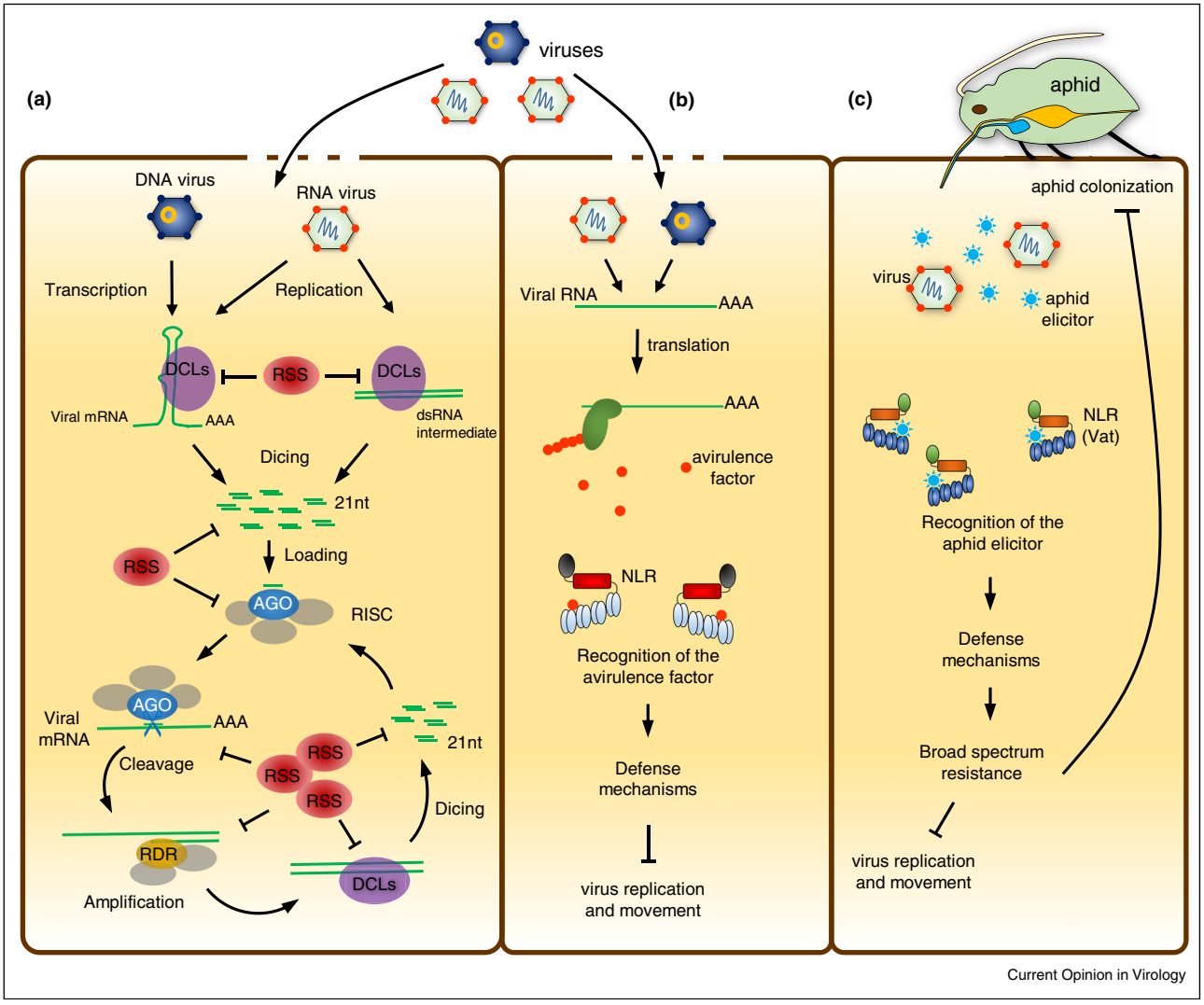
An important feature shared by plant viruses is their efficient movement from host to host. This virus transmission is a vital step in the biological cycle of viruses because it ensures their maintenance, survival and spread. The virus transmission cycle involves a continuum of processes, acquisition of the virus when the vector feeds on a virus-infected plant, stable retention and transport of the virus within the vector, and inoculation of the retained virus into a new host plant during a subsequent feeding. Most plant viruses (76%) are transmitted by a diverse array of vectors including insects, nematodes and fungi. Many of these vectors are plant pests, and their association with plants

makes them ideal agents for efficient local and long-distance virus spread. By far, insects, the most common plant virus vectors, transmit the majority of described plant viruses, and of these, hemipteran insects transmit 55% of the vectored viruses [5]. In most cases, viruses of a given taxon have a specific type of insect vector. For example, viruses of the genus *Potyvirus* and *Begomovirus* are solely transmitted by aphids and whiteflies, the most economically important insect vectors, respectively.

Antiviral RNA silencing defense

Once infected, plants rely on elaborate antiviral immune arsenal to defend themselves against the invading viruses. One of the immediate antiviral defense plant viruses encountered when invading a host is the RNA silencing (Figure 1a) [6]. RNA silencing, also called RNA interference (RNAi), is an evolutionary conserved and sequence-specific mechanism that directly defends host cells against foreign nucleic acids such as viruses and transposable elements [7]. This defense is triggered by double-stranded RNA molecules (dsRNA). Most plant viruses have RNA genomes that replicate through dsRNA intermediates by viral RNA-dependant RNA polymerases (RDRs) or contain double-stranded secondary structures. These viral dsRNAs are processed by Dicer-like (DCL) enzymes into virus-derived small RNAs (vsRNAs) that

Figure 1



Mechanisms of plant resistance to viruses. **(a)** Antiviral RNA silencing in plants and its suppression by virus-encoded RNA silencing suppressors (RSSs). RNA silencing is initiated by the recognition of viral dsRNAs or partially double strand hairpin RNAs, which are processed to vsiRNAs. (**b**) NLR-mediated plant resistance. Following entry into a host cell, viral effectors are expressed from the virus genome. Specific plant NLR genes interact (directly or indirectly) with these effectors to trigger virus resistance. (**c**) Model for *Vat*-mediated resistance involving separate recognition and response phases. In the *A. gossypii* resistant plant, the Vat-NLR recognizes an elicitor molecule from the aphid. This recognition phase induces local resistance mechanisms that inhibit aphid colonization and replication and movement of viruses transmitted by the same aphid.

are uploaded into the RNA-induced silencing complex (RISC) and used to guide the silencing of the viral genome [8–10]. The antiviral RNA silencing response acts against all RNA and DNA viruses, but since DNA viruses do not replicate through dsRNA intermediates, precursors of vsRNAs could potentially be formed by antisense transcription, RDR activity or from secondary structures of viral RNAs (Figure 1a) [11,12]. To resist virus infections locally and systemically, plants generate secondary vsRNAs, the mobile silencing signal that spreads from the site of initiation to the surrounding tissues and over long distances via the plasmodesmata and the phloem. This non-cell autonomous process primes RNA silencing in non-infected cells and depends on host RDRs proteins [13,14].

To escape the antiviral RNA silencing defense, almost all types of plant viruses have evolved RNA silencing suppressors (RSSs). Although, RSSs are highly diverse in sequence, structure and involved in a number of basic viral functions (replication, movement and encapsidation), their modes of action can be classified into three classes: first, binding to dsRNA resulting in inhibition of vsRNA production by DCL proteins; second, sequestration of sRNA duplexes and interfering with RISC assembly and cell-to-cell movement of vsRNA; and third, direct targeting of effectors or processing factors leading to their inhibition or destabilization (Figure 1a) [15••–19]. Because of the central role of RNA silencing in plant development, defense and adaptation to stress, specific strategies have been developed by the host to neutralize the effects of RSSs and stimulate the defense reactions [10]. Among the counter-counterdefense responses, the plant hosts use resistance (R) proteins to guard the integrity of RNA silencing components and to either directly or indirectly recognize RSSs and finally induce hypersensitive response (HR) [20–23].

Hypersensitive response

One common feature of the host immune resistance is the rapid induction of programmed cell death at the site of pathogen invasion and at the immediately surrounding cells. This symptomatic manifestation is called Hypersensitive Response (HR) [24]. The HR is triggered by a wide variety of pathogens, including viruses, to prevent pathogen spread in the plant [24]. Hypersensitive reactions are initiated by the recognition of the pathogen-encoded avirulence factor (Avr) by the plant host R genes (Figure 1b) [25•]. The Avr/R protein interactions may trigger a mitogen-activated protein kinase (MAPK) signaling cascade and lead to a fast accumulation of reactive oxygen species (ROS) and defense hormones, salicylic acid (SA) and jasmonic acid (JA). At the cellular level, HR is associated with calcium ion influx, callose deposition at the plasmodesmata, modification of the membrane permeability and a drastic transcriptional reprogramming leading to the expression of Pathogenesis Related (PR) genes [26–29].

Although, the HR cell death and the resistance response are closely associated, increasing evidence shows that these two defense components can be physiologically, genetically and temporally uncoupled. Among the supporting examples, the interaction of the potato *Rx1* resistance gene with the *Potato virus X* (PVX) capsid protein (CP) inhibits PVX replication independently of the CP-triggered HR cell death [30••].

The vast majority of the cloned dominant R genes encode nucleotide-binding leucine-rich repeat (NB-LRRs or NLRs) proteins that recognize the Avr factor through a 'gene-for-gene' interaction (Table 1) [31]. NLR genes usually confer narrow resistance spectrum, restricted to a single pathogen and usually to a limited number of strains. Irrespective of the pathogen they perceive, canonical plant NLRs proteins consist of trimodular domains: first, the N-terminal Toll-interleukine-1 receptor (TIR) or coiled-coil (CC) domains that define the two main classes of R proteins: TIR-NLRs or CC-NLRs, (ii) the central nucleotide binding (NB) domain and (iii) the leucine-rich repeat (LRR) domain at the C-terminal end [25•,32,33••,34]. Strong genetic evidence supports that the LRR domain determines the pathogen recognition specificity and that this domain is under diversifying selection pressure to evolve new pathogen recognition specificity [33••,35••,36].

NLRs proteins function as molecular switches, shifting between a constitutively inactive 'off' conformation and an active 'on' state after the recognition of the pathogen. To prevent unnecessary activation of the NLR-mediated responses, intramolecular interactions between the LRR domain folded back across the NB domain and the N-terminal dimerization, TIR or CC, domain maintain the NLRs proteins into the inactive 'off' mode [37••]. Recent studies on the potato *Rx1* gene that encodes a CC-NB-LRR protein and mediates resistance to PVX demonstrate that intramolecular CC-NB and NB-LRR domain interactions keep Rx1 protein in an inactive state [38••,39–41].

The translation of the pathogen recognition into an efficient resistance response is attributed to the NB domain [42]. Within this domain, the P-loop and the MHD motifs are essential for the NLR function. Substitutions in the P-loop inactivate the NLRs proteins whereas mutations of the aspartic acid (D) in the MHD motif render many NLRs proteins autoactive initiating the resistance response in the absence of pathogen or avirulence protein [38••,43].

R gene-mediated resistance to aphid infestation and aphid-transmitted viruses

Aphids are the major virus-transmitting pests of plants. Besides vectoring numerous devastating viruses, aphids

Table 1

NLRs genes cloned for a dominant resistance against plant viruses

Plant species	*R* gene (NRL type)	Virus recognized	Avirulence factor	References
Nicotiana glutinosa	*N* (TIR-NLR)	TMV	p50	[49••]
Solanum tuberosum	*Rx1* (CC-NLR)	PVX	CP	[30••]
	Rx2 (CC-NLR)	PVX	CP	[50]
	Y-1 (TIR-NLR)	PVY	Unknown	[51]
Arabidopsis thaliana	*HRT* (CC-NLR)	TCV	CP	[52]
	RCY1 (CC-NLR)	CMV	CP	[53]
Solanum peruvianum	*Sw5b* (CC-NLR)	TSWV	NSm	[54]
Solanum lycopersicum	*Tm-2* (CC-NLR)	TMV, ToMV	30 kDa MP	[55,56]
Glycine max	*Rsv1* (CC-NLR)	SMV	P3 + HC-Pro	[57,20]
Phaseolus vulgaris	*I* (TIR-NLR)	BCMV, BNMV, BICMV, AzMV, CABMV, PWV, SMV, ThPV, WMV, ZYMV	Unknown	[58]
	PvVTT1 (TIR-NLR)	BDMV	BV1 (NSP)	[59]
	PvCMR1(TIR-NLR)	CMV	2a	[60]
Poncirus trifoliata	*Ctv* (CC-NLR)	CTV	Unknown	[61,62]
Capsicum annuum	L^{1-4} (CC-NLR)	TMV, ToMV, TMGMV, BPeMV, PaMMV, ObPV, PMMoV	CP	[63]
Brassica campestris	*BcTuR3* (TIR-NLR)	TuMV	Unknown	[64]
Brassica rapa	*TuRB07 (CC-NLR)*	TuMV	Unknown	[65]
Vigna mungo	*CYR1* (CC-NLR)	MYMV	CP	[66]
Cucumis melo	Pvr^{1-2} (TIR-NLR)	PRSV	Unknown	[34]
	Vat (CC-NLR)		Unknown	[33••]

CC, coiled-coil; TIR, Toll/interleukin 1 receptor; CP, Coat Protein; MP, Movement Protein; NSP, Nuclear Shuttle Protein; AzMV, *Azuki mosaic virus*; BCMV, *Bean common mosaic virus*; BDMV, *Bean dwarf mosaic virus*; BICMV, *Blackeye cowpea mosaic virus*; BNMV, *Bean necrotic mosaic virus*; BPeMV, *Bell pepper mottle virus*; CABMV, *Cowpea aphid-borne mosaic virus*; CMV, *Cucumber mosaic virus*; CTV, *Citrus tristeza virus*; MYMV, *Mungbean yellow mosaic virus*; ObPV, *Obuda pepper virus*; PaMMV, *Paprika mild mottle virus*; PMMoV, *Pepper mild mottle virus*; PRSV, *Papaya ringspot virus*; PVX, *Potato virus X*; PVY, *Potato virus Y*; PWV, *Passionfruit woodiness virus*; SMV, *Soybean mosaic virus*; TCV, *Turnip crinkle virus*; ThPV, *Thailand passiflora virus*; TMGMV, *Tobacco mild green mosaic virus*; TMV, *Tobacco mosaic virus*; ToMV, *Tomato mosaic virus*; TSWV, *Tomato spotted wilt virus*; TuMV, *Turnip mosaic virus*; WMV, *Watermelon mosaic virus*; ZYMV, *Zucchini yellow mosaic virus*.

also affect plant health by directly feeding on phloem sap [5,44]. Recently, the isolation of the dominant *Virus aphid transmission* (*Vat*) gene from melon, *Cucumis melo*, provides novel insights into the double resistance to the aphid, *Aphis gossypii*, infestation and *A. gossypii*-vectored viruses [33••] The *Vat* gene encodes a NLR protein with an unusual conserved LRR repeat [33••]. *Vat* confers resistance to the aphid-transmitted *Cucumber mosaic virus (CMV)* and to several aphid-transmitted potyviruses, although these viruses have very distinct infection strategies [45]. These viruses are vectored within the saliva of *A. gossypii* and the *Vat*-mediated virus resistance is observed only if host plants harboring *Vat* gene are infected by an avirulent strain of *A. gossypii*. When plants are infected mechanically or by another aphid species, virus infection occurs on either the *Vat* resistant and non-*Vat* susceptible plants [33••]. The *Vat* gene example is an elegant illustration of the two-steps process, a specific recognition of an aphid, *A. gossypii*, and a triggered broad-spectrum resistance that blocks virus infection (Figure 1c). However, questions regarding the identity of the aphid avirulence factors necessary to induce the Vat-mediated resistance still unresolved.

Conclusions and perspectives

Nowadays, virus disease management consists mainly in agricultural practices such as the destruction of infected plants, the eradication of plants reservoir and the use of pesticide to limit the population of vectors. Another powerful mean to control virus infection is the use of disease resistant genes in breeding programs. Unfortunately R genes do not exist against all the devastating viruses and when available their introgression in high yielding varieties is tedious. Among the hurdles, the difficulty to cross elite lines with non-cultivated plants and the linkage drag of unwanted loci that reduce the agronomic fitness of the crop. Moreover, R genes are in many cases overcome by resistant breaking strains.

In the post genomic era, one would expect to use gene editing tools to design a resistance gene against an important viral function. Such function should have stringent constraint so that its mutation probably affects the fitness of the virus. Until recently this was a dream. The development of tools such as the TALENs (Transcription Activator-Like Effector Nucleases) and the CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) have opened wide the possibility to engineer a new generation of resistance genes. In this scenario, plant virologist will bring new insight on how the virus highjacks the plant machinery to identify key viral protein domains that the virus can not easily modify. In the case of active resistance, molecular biologist will modify existing R genes so that new virus variant will be recognized by the host plant and eradicated by the plant immune system. In the case of passive resistance,

induced mutation in a plant gene will create plant protein that will not penalize the plant growth but in the same time is not recognized by the virus as a host factor.

The current progress in gene editing will no doubt boost the development of genetic engineering of R genes. If we succeed in engineering such R genes, such approach is likely to provide a high level of protection, and because they are on the basis of a plant's own defense arsenal, they are likely to provide durable resistance as well. The pioneer work on in artificial evolution of R genes in the pathosystem *Rx*-Potex virus has set the ground for such work. Rx mutants, with enhanced recognition of *Rx*-resistant breaking strains of PVX or other viruses such as *Poplar mosaic virus* (PopMV) [46••,47•], were identified. Similarly, for recessive resistance, induced mutations in translational initiation factor such as *eIF4E* have led to *Potato virus Y* (PVY) resistance in tomato [48•].

Acknowledgements

We thank the editors for the invitation to write this review. We also thank Judit Szecsi, Mohammed Bendahmane and Victoria Gomez-Roldan for comments and careful reading of the manuscript. This work was supported by the Plant Biology and Breeding department in INRA, the grants Program Saclay Plant Sciences (SPS, ANR-10-LABX-40), IDEX Paris-Saclay (Lidex-3P, ANR-11-IDEX-0003-02) and the European Research Council (ERC-SEXYPARTH).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- • of special interest

1. Dangl JL, Horvath DM, Staskawicz BJ: **Pivoting the plant immune system from dissection to deployment**. *Science* 2013, **341**:746-751.

2. Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR, Daszak P: **Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers**. *Trends Ecol Evol* 2004, **19**:535-544.

3. •• Hull R: *Plant Virology*. edn 5. Academic Press; 2013.

Essential text book in plant virology

4. Legg JP, Owor B, Sseruwagi P, Ndunguru J: **Cassava mosaic virus disease in East and Central Africa: epidemiology and management of a regional pandemic**. *Adv Virus Res* 2006, **67**:355-418.

5. Hogenhout SA, Ammar el D, Whitfield AE, Redinbaugh MG: **Insect vector interactions with persistently transmitted viruses**. *Annu Rev Phytopathol* 2008, **46**:327-359.

6. Ding SW, Voinnet O: **Antiviral immunity directed by small RNAs**. *Cell* 2007, **130**:413-426.

7. Voinnet O: **Origin, biogenesis, and activity of plant microRNAs**. *Cell* 2009, **136**:669-687.

8. Sharma N, Sahu PP, Puranik S, Prasad M: **Recent advances in plant-virus interaction with emphasis on small interfering RNAs (siRNAs)**. *Mol Biotechnol* 2013, **55**:63-77.

9. Wang XB, Jovel J, Udomporn P, Wang Y, Wu Q, Li WX, Gasciolli V, Vaucheret H, Ding SW: **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* 2011, **23**:1625-1638.

10. Pumplin N, Voinnet O: **RNA silencing suppression by plant pathogens: defence, counter-defence and counter-counter-defence**. *Nat Rev Microbiol* 2013, **11**:745-760.

11. Aregger M, Borah BK, Seguin J, Rajeswaran R, Gubaeva EG, Zvereva AS, Windels D, Vazquez F, Blevins T, Farinelli L *et al.*: **Primary and secondary siRNAs in geminivirus-induced gene silencing**. *PLoS Pathog* 2012, **8**:e1002941.

12. Blevins T, Rajeswaran R, Shivaprasad PV, Beknazariants D, Si-Ammour A, Park HS, Vazquez F, Robertson D, Meins F Jr, Hohn T *et al.*: **Four plant Dicers mediate viral small RNA biogenesis and DNA virus induced silencing**. *Nucleic Acids Res* 2006, **34**:6233-6246.

13. Wang XB, Wu Q, Ito T, Cillo F, Li WX, Chen X, Yu JL, Ding SW: **RNAi-mediated viral immunity requires amplification of virus-derived siRNAs in *Arabidopsis thaliana***. *Proc Natl Acad Sci U S A* 2010, **107**:484-489.

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

15. •• Burgyan J, Havelda Z: **Viral suppressors of RNA silencing**. *Trends Plant Sci* 2011, **16**:265-272.

Excellent review describing how viruses use repressors of gene silencing to fight against antiviral RNA silencing mechanisms.

16. Chiu MH, Chen IH, Baulcombe DC, Tsai CH: **The silencing suppressor P25 of Potato virus X interacts with Argonaute1 and mediates its degradation through the proteasome pathway**. *Mol Plant Pathol* 2010, **11**:641-649.

17. Giner A, Lakatos L, Garcia-Chapa M, Lopez-Moya JJ, Burgyan J: **Viral protein inhibits RISC activity by argonaute binding through conserved WG/GW motifs**. *PLoS Pathog* 2010, **6**:e1000996.

18. Derrien B, Baumberger N, Schepetilnikov M, Viotti C, De Cillia J, Ziegler-Graff V, Isono E, Schumacher K, Genschik P: **Degradation of the antiviral component ARGONAUTE1 by the autophagy pathway**. *Proc Natl Acad Sci U S A* 2012, **109**:15942-15946.

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

20. Wen RH, Khatabi B, Ashfield T, Saghai Maroof MA, Hajimorad MR: **The HC-Pro and P3 cistrons of an avirulent Soybean mosaic virus are recognized by different resistance genes at the complex Rsv1 locus**. *Mol Plant Microbe Interact* 2013, **26**:203-215.

21. Choi CW, Qu F, Ren T, Ye X, Morris TJ: **RNA silencing-suppressor function of Turnip crinkle virus coat protein cannot be attributed to its interaction with the Arabidopsis protein TIP**. *J Gen Virol* 2004, **85**:3415-3420.

22. Angel CA, Hsieh YC, Schoelz JE: **Comparative analysis of the capacity of tombusvirus P22 and P19 proteins to function as avirulence determinants in Nicotiana species**. *Mol Plant Microbe Interact* 2011, **24**:91-99.

23. de Ronde D, Butterbach P, Lohuis D, Hedil M, van Lent JW, Kormelink R: **Tsw gene-based resistance is triggered by a functional RNA silencing suppressor protein of the Tomato spotted wilt virus**. *Mol Plant Pathol* 2013, **14**:405-415.

24. oll NS, Epple P, Dangl JL: **Programmed cell death in the plant immune system**. *Cell Death Differ* 2011, **18**:1247-1256.

25. • Moffett P: **Mechanisms of recognition in dominant R gene mediated resistance**. *Adv Virus Res* 2009, **75**:1-33.

Good review of how NB-LRR proteins interact with Avr proteins.

26. Culver JN, Padmanabhan MS: **Virus-induced disease: altering host physiology one interaction at a time**. *Annu Rev Phytopathol* 2007, **45**:221-243.

27. Carr JP, Lewsey MG, Palukaitis P: **Signaling in induced resistance**. *Adv Virus Res* 2010, **76**:57-121.

28. Pallas V, Garcia JA: **How do plant viruses induce disease? Interactions and interference with host components**. *J Gen Virol* 2011:92:2691-2705:.

29. Mandadi KK, Scholthof KB: **Characterization of a viral synergism in the monocot Brachypodium distachyon reveals distinctly altered host molecular processes associated with disease**. *Plant Physiol* 2012, **160**:1432-1452.

30. •• Bendahmane A, Kanyuka K, Baulcombe DC: **The Rx gene from potato controls separate virus resistance and cell death responses**. *Plant Cell* 1999, **11**:781-792.
The authors show that R genes can lead to resistance without cell death and that cell death and virus arrest are separate disease resistance responses in plants.

31. Flor HH: **Current status of the gene-for-gene concept**. *Annu Rev Phytopathol* 1971, **9**:275-296.

32. Gururani MA, Venkatesh J, Upadhyaya CP, Nookaraju A, Pandey SK, Park SW: **Plant disease resistance genes: current status and future directions**. *Physiol Mol Plant Pathol* 2012, **78**:51-65.

33. •• Dogimont C, Chovelon V, Pauquet J, Boualem A, Bendahmane A: **The Vat locus encodes for a CC-NBS-LRR protein that confers resistance to *Aphis gossypii* infestation and *A. gossypii*-mediated virus resistance**. *Plant J* 2014, **80**:993-1004.
The paper shows that R genes mediated resistance could be divided on two phases, recognition and response. The recognition phase involves the interaction of an elicitor molecule from the aphid and Vat from the plant. The response phase is not specific and blocks both aphid infestation and virus infection.

34. Brotman Y, Normantovich M, Goldenberg Z, Zvirin Z, Kovalski I, Stovbun N, Doniger T, Bolger AM, Troadec C, Bendahmane A *et al.*: **Dual resistance of melon to Fusarium oxysporum races 0 and 2 and to Papaya ring-spot virus is controlled by a pair of head-to-head-oriented NB-LRR genes of unusual architecture**. *Mol Plant* 2013, **6**:235-238.

35. •• Jones JD, Dangl JL: **The plant immune system**. *Nature* 2006, **444**:323-329.
Excellent review describing, through the zigzag model, the quantitative and the qualitative output of the plant immune system.

36. Bernoux M, Ellis JG, Dodds PN: **New insights in plant immunity signaling activation**. *Curr Opin Plant Biol* 2011, **14**:512-518.

37. •• Takken FL, Goverse A: **How to build a pathogen detector: structural basis of NB-LRR function**. *Curr Opin Plant Biol* 2012, **15**:375-384.
Excellent review about how NB-LRRs mediate recognition of pathogen-derived effectors molecules and subsequently activate host defense.

38. •• Bendahmane A, Farnham G, Moffett P, Baulcombe DC: **Constitutive gain-of-function mutants in a nucleotide binding site-leucine rich repeat protein encoded at the Rx locus of potato**. *Plant J* 2002, **32**:195-204.
This paper shows how R genes can lead to cell death in the absence of elicitors.

39. Moffett P, Farnham G, Peart J, Baulcombe DC: **Interaction between domains of a plant NBS-LRR protein in disease resistance-related cell death**. *EMBO J* 2002, **21**:4511-4519.

40. Lukasik E, Takken FL: **STANDing strong, resistance proteins instigators of plant defence**. *Curr Opin Plant Biol* 2009, **12**:427-436.

41. Slootweg EJ, Spiridon LN, Roosien J, Butterbach P, Pomp R, Westerhof L, Wilbers R, Bakker E, Bakker J, Petrescu AJ *et al.*: **Structural determinants at the interface of the ARC2 and leucine-rich repeat domains control the activation of the plant immune receptors Rx1 and Gpa2**. *Plant Physiol* 2013, **162**:1510-1528.

42. Eitas TK, Dangl JL: **NB-LRR proteins: pairs, pieces, perception, partners, and pathways**. *Curr Opin Plant Biol* 2010, **13**:472-477.

43. Stirnweis D, Milani SD, Jordan T, Keller B, Brunner S: **Substitutions of two amino acids in the nucleotide-binding site domain of a resistance protein enhance the hypersensitive response and enlarge the PM3 F resistance spectrum in wheat**. *Mol Plant Microbe Interact* 2014, **27**:265-276.

44. Ng JC, Perry KL: **Transmission of plant viruses by aphid vectors**. *Mol Plant Pathol* 2004, **5**:505-511.

45. Lecoq H, Desbiez C: **Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture**. *Adv Virus Res* 2012, **84**:67-126.

46. •• Farnham G, Baulcombe DC: **Artificial evolution extends the spectrum of viruses that are targeted by a disease-resistance gene from potato**. *Proc Natl Acad Sci U S A* 2006, **103**:18828-18833.
One of the first examples of engineering of broader spectrum of disease-resistance specificity by mutagenesis.

47. • Harris CJ, Slootweg EJ, Goverse A, Baulcombe DC: **Stepwise artificial evolution of a plant disease resistance gene**. *Proc Natl Acad Sci U S A* 2013, **110**:21189-21194.
The work describes artificial evolution of NB-LRR disease resistance genes. The engineered alleles enhanced the activation sensitivity rather than the recognition phase of a NB-LRR, Rx, which led to broad-spectrum resistance against PVX strains and PopMV.

48. • Piron F, Nicolai M, Minoia S, Piednoir E, Moretti A, Salgues A, Zamir D, Caranta C, Bendahmane A: **An induced mutation in tomato eIF4 E leads to immunity to two potyviruses**. *PLoS ONE* 2010, **5**:e11313.
A good example of engineered resistant plant by knocking a host factor, in this case required for translation of viral RNA.

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

50. Bendahmane A, Querci M, Kanyuka K, Baulcombe DC: **Agrobacterium transient expression system as a tool for the isolation of disease resistance genes: application to the Rx2 locus in potato**. *Plant J* 2000, **21**:73-81.

51. Vidal S, Cabrera H, Andersson RA, Fredriksson A, Valkonen JP: **Potato gene Y-1 is an N gene homolog that confers cell death upon infection with potato virus Y**. *Mol Plant Microbe Interact* 2002, **15**:717-727.

52. Cooley MB, Pathirana S, Wu HJ, Kachroo P, Klessig DF: **Members of the Arabidopsis HRT/RPP8 family of resistance genes confer resistance to both viral and oomycete pathogens**. *Plant Cell* 2000, **12**:663-676.

53. Takahashi H, Miller J, Nozaki Y, Takeda M, Shah J, Hase S, Ikegami M, Ehara Y, Dinesh-Kumar SP, Sukamto: RCY1: **an *Arabidopsis thaliana* RPP8/HRT family resistance gene, conferring resistance to cucumber mosaic virus requires salicylic acid, ethylene and a novel signal transduction mechanism**. *Plant J* 2002, **32**:655-667.

54. Brommonschenkel SH, Frary A, Frary A, Tanksley SD: **The broad-spectrum tospovirus resistance gene Sw-5 of tomato is a homolog of the root-knot nematode resistance gene Mi**. *Mol Plant Microbe Interact* 2000, **13**:1130-1138.

55. Lanfermeijer FC, Dijkhuis J, Sturre MJ, de Haan P, Hille J: **Cloning and characterization of the durable tomato mosaic virus resistance gene Tm-2(2) from *Lycopersicon esculentum***. *Plant Mol Biol* 2003, **52**:1037-1049.

56. Lanfermeijer FC, Warmink J, Hille J: **The products of the broken Tm-2 and the durable Tm-2(2) resistance genes from tomato differ in four amino acids**. *J Exp Bot* 2005, **56**:2925-2933.

57. Hayes AJ, Jeong SC, Gore MA, Yu YG, Buss GR, Tolin SA, Maroof MA: **Recombination within a nucleotide-binding-site/leucine-rich-repeat gene cluster produces new variants conditioning resistance to soybean mosaic virus in soybeans**. *Genetics* 2004, **166**:493-503.

58. Vallejos CE, Astua-Monge G, Jones V, Plyler TR, Sakiyama NS, Mackenzie SA: **Genetic and molecular characterization of the I locus of *Phaseolus vulgaris***. *Genetics* 2006, **172**:1229-1242.

59. Seo YS, Jeon JS, Rojas MR, Gilbertson RL: **Characterization of a novel Toll/interleukin-1 receptor (TIR)-TIR gene differentially expressed in common bean (Phaseolus vulgaris cv. Othello) undergoing a defence response to the geminivirus Bean dwarf mosaic virus**. *Mol Plant Pathol* 2007, **8**:151-162.

60. Seo YS, Rojas MR, Lee JY, Lee SW, Jeon JS, Ronald P, Lucas WJ, Gilbertson RL: **A viral resistance gene from common bean functions across plant families and is up-regulated in a non-virus-specific manner**. *Proc Natl Acad Sci U S A* 2006, **103**:11856-11861.

61. Yang ZN, Ye XR, Molina J, Roose ML, Mirkov TE: **Sequence analysis of a 282-kilobase region surrounding the citrus Tristeza virus resistance gene (Ctv) locus in *Poncirus trifoliata* L. Raf.**. *Plant Physiol* 2003, **131**:482-492.

62. Rai M: **Refinement of the *Citrus tristeza* virus resistance gene (Ctv) positional map in *Poncirus trifoliata* and generation of transgenic grapefruit (*Citrus paradisi*) plant lines with candidate resistance genes in this region**. *Plant Mol Biol* 2006, **61**:399-414.

63. Tomita R, Sekine KT, Mizumoto H, Sakamoto M, Murai J, Kiba A, Hikichi Y, Suzuki K, Kobayashi K: **Genetic basis for the hierarchical interaction between Tobamovirus spp. and L resistance gene alleles from different pepper species**. *Mol Plant Microbe Interact* 2011, **24**:108-117.

64. Ma J, Hou X, Xiao D, Qi L, Wang F, Sun F, Wang Q: **Cloning and characterization of the *BcTuR3* gene related to resistance to *Turnip mosaic virus* (TuMV) from non-heading Chinese cabbage**. *Plant Mol Biol Rep* 2010, **28**:588-596.

65. Jin M, Lee SS, Ke L, Kim JS, Seo MS, Sohn SH, Park BS, Bonnema G: **Identification and mapping of a novel dominant resistance gene, TuRB07 to Turnip mosaic virus in Brassica rapa**. *Theor Appl Genet* 2014, **127**:509-519.

66. Maiti S, Paul S, Pal A: **Isolation, characterization, and structure analysis of a non-TIR-NBS-LRR encoding candidate gene from MYMIV-resistant Vigna mungo**. *Mol Biotechnol* 2012, **52**:217-233.