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Review

Exploitation of natural genetic diversity to study plant–virus interactions: what can we learn from *Arabidopsis thaliana*?

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SUMMARY

The development and use of cultivars that are genetically resistant to viruses is an efficient strategy to tackle the problems of virus diseases. Over the past two decades, the model plant *Arabidopsis thaliana* has been documented as a host for a broad range of viral species, providing access to a large panel of resources and tools for the study of viral infection processes and resistance mechanisms. Exploration of its natural genetic diversity has revealed a wide range of genes conferring virus resistance. The molecular characterization of some of these genes has unveiled resistance mechanisms distinct from those described in crops. In these respects, *Arabidopsis* represents a rich and largely untapped source of new genes and mechanisms involved in virus resistance. Here, we review the current status of our knowledge concerning natural virus resistance in *Arabidopsis*. We also address the impact of environmental conditions on *Arabidopsis*–virus interactions and resistance mechanisms, and discuss the potential of applying the knowledge gained from the study of *Arabidopsis* natural diversity for crop improvement.

INTRODUCTION

Virus diseases are a significant threat to crop production because they can cause high losses in yield and quality and no direct countermeasures are available to fight these pathogens. Among the methods available to control viral infections, the most effective and sustainable approach is through the deployment of genetic resistance targeted either directly against viruses or indirectly against their vectors. Recently, there have been dramatic advances in our understanding of the molecular nature and mechanisms associated with natural virus resistance genes (Maule *et al*., 2007). However, the use of virus resistance genes, although successful, is hindered because they are not always available in the natural diversity of crop plants. There is consequently a need to identify novel resistance genes and mechanisms and to exploit technical advances that will ease the introduction of these genes and pathways into breeding programmes.

The finding that many viruses, including some of the most common and destructive ones for widely grown crops, are able to efficiently infect *Arabidopsis thaliana* under experimental settings greatly stimulated interest in using this species to decipher plant–virus interactions and resistance mechanisms. *Arabidopsis* displays a wide range of phenotypic and genetic variation that can be efficiently exploited by analysing collections of stock centre accessions, which are available to the plant research community (Borevitz *et al*., 2007; Koornneef *et al*., 2004; McKhann *et al*., 2004; Platt *et al*., 2010). This feature, combined with the practical advantages of its small size and short life cycle, allows the rapid and efficient exploration of its natural variation to identify resistance mechanisms. Importantly, the availability of a simple, small and completely sequenced genome, together with access to a wide array of genomic and molecular resources and the ability to easily transform plants render *Arabidopsis* particularly amenable to deciphering the genetic basis and molecular mechanisms underlying resistance phenotypes (Koornneef and Meinke, 2010; Leonelli, 2007).

The rationale to build on the advances in *Arabidopsis* and to exploit this knowledge for crop improvement comes from several studies that support a great deal of conservation between *Arabidopsis* and crop species in the plant factors mediating interactions with viruses. For instance, *Arabidopsis* T-DNA mutants lacking the eukaryotic translation initiation factor eIF4E present a similar resistance phenotype against RNA viruses from the genus *Potyvirus* as that observed in crops showing naturally occurring amino acid changes in eIF4E proteins (Le Gall *et al*., 2011). The *TOBAMOVIRUS MULTIPLICATION 1* (*TOM1*) and *TOM3* genes, which play an essential role in the replication of *Tobacco mosaic virus* (TMV), constitute another significant example. *TOM1* and *TOM3* were isolated in a screen for *Arabidopsis* mutants defective for infection by TMV (Yamanaka *et al*., 2002), and simultaneous RNA interference against both the *TOM1* and *TOM3* orthologs from *Nicotiana tabacum* was shown to result in nearly complete inhibition of TMV multiplication in tobacco (Asano *et al*., 2005).

This article presents an overview of the features that make *Arabidopsis* a uniquely well-suited system in which to study host–

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virus interactions, with particular emphasis on the exploration of its natural genetic diversity to decipher the mechanisms controlled by resistance genes. We also underline the importance of environmental factors that influence *Arabidopsis* responses to viral pathogens, and consider future prospects and technical advances that will ease the exploitation of novel genes and resistance mechanisms for crop improvement.

ARABIDOPSIS IS A COMPATIBLE HOST FOR A WIDE RANGE OF VIRUSES

The development of various artificial methods for virus inoculation (e.g. mechanical inoculation with virus-infected leaf sap or purified virus preparations, *Agrobacterium*- or biolistic-mediated transfer of cloned virus genomes) has established *Arabidopsis* as a compatible host for numerous plant viruses. To date, approximately 40 viral species belonging to 18 genera have been shown to infect at least one accession of *Arabidopsis* under experimental conditions (Table 1). The vast majority of these viruses (30 of 40) are positive-stranded (+) RNA viruses and include species from many genera, including *Alfamo*-, *Bromo*-, *Carmo*-, *Como*-, *Cucumo*-, *Nepo*-, *Polero*-, *Poty*-, *Potex*-, *Tobamo*-, *Tobra*- and *Tymovirus*. In addition, *Arabidopsis* is a host for the negative stranded (–) RNA virus *Tomato spotted wilt virus* (genus *Tospovirus*), as well as for several viruses with DNA-encoded genomes, including single-stranded DNA viruses belonging to the genera *Begomo*-, *Curto*-, *Nano*- and *Mastrevirus* and the double-stranded DNA virus *Cauliflower mosaic virus* (CaMV; genus *Caulimovirus*).

Our knowledge of the ability of these viruses to infect *Arabidopsis* under natural conditions remains sparse. Only one study has addressed this issue, by monitoring the occurrence of five viral species, CaMV, *Cucumber mosaic virus* (CMV), *Turnip yellow*

Table 1 Viral species infecting *Arabidopsis thaliana*.

Genome	Genus	Virus	Reference
(+) ssRNA	*Alfamovirus*	*Alfalfa mosaic virus* (*AMV*)	Balasubramaniam *et al*. (2006)
	Bromovirus	*Brome mosaic virus* (*BMV*)	Dzianott and Bujarski (2004)
		Cassia yellow blotch virus (*CYBV*)	Iwahashi *et al*. (2005)
		Cow pea chlorotic mottle virus (*CCMV*)	Fujisaki *et al*. (2003)
		Melandrium yellow fleck virus (*MYFV*)	Narabayashi *et al*. (2009)
		Spring beauty latent virus (*SBLV*)	Fujisaki *et al*. (2003)
	Carmovirus	*Cardamine chlorotic fleck virus* (*CCFV*)	Skotnicki *et al*. (1993)
		***Turnip crinkle virus* (*TCV*)**	Li and Simon (1990)
	Comovirus	*Turnip ringspot virus* (*TuRSV*)	Rajakaruna and Khandekar (2007)
	Cucumovirus	***Cucumber mosaic virus* (*CMV*)**	Takahashi *et al*. (1994)
	Nepovirus	*Arabis mosaic virus* (*ArMV*)	Martinez-Herrera *et al*. (1994)
		Tobacco ringspot virus (*TRSV*)	Lee *et al*. (1996)
		Cherry leaf roll virus (*CLRV*)	Rumbou *et al*. (2009)
	Polerovirus	*Beet mild yellowing virus* (*BMYV*)	Stevens *et al*. (2005)
		Beet western yellow virus (*BWYV*)	Pazhouhandeh *et al*. (2006)
		Cucurbit aphid-borne yellow virus (*CABYV*)	
		Turnip yellow virus (*TuYV*)	Stevens *et al*. (2005)
	Potyvirus	*Lettuce mosaic virus* (*LMV*)	Revers *et al*. (2003)
		Plum pox virus (*PPV*)	Decroocq *et al*. (2006)
		Potato virus Y (*PVY*)	Whitham *et al*. (2000)
		Tobacco etch virus (*TEV*)	Mahajan *et al*. (1998)
		Tobacco vein mottling virus (*TVMV*)	Whitham *et al*. (2000)
		***Turnip mosaic virus* (*TuMV*)**	Martinez-Herrera *et al*. (1994)
	Potexvirus	*Plantago asiatica mosaic virus* (*PlAMV*)	Yamaji *et al*. (2012)
	Tobamovirus	*Oilseed rape mosaic virus* (*ORMV*)	Aguilar *et al*. (1996)
		Tobacco mosaic virus (*TMV*)	Ishikawa *et al*. (1991)
		Turnip vein clearing virus (*TVCV*)	Lartey *et al*. (1997)
	Tobravirus	*Tobacco rattle virus* (*TRV*)	Donaire *et al*. (2008)
		Pepper ringspot virus (*PepRSV*)	Jaubert *et al*. (2011)
	Tymovirus	***Turnip yellow mosaic virus* (*TYMV*)**	Martinez-Herrera *et al*. (1994)
(–) ssRNA	*Tospovirus*	*Tomato spotted wilt virus* (*TSWV*)	German *et al*. (1995)
ssDNA	*Begomovirus*	*Cabbage leaf curl virus* (*CaLCuV*)	Hill *et al*. (1998)
		Cleome leaf crumple virus (*ClLCrV*)	Paprotka *et al*. (2010)
		Euphorbia mosaic virus (*EuMV*)	
		Sri Lankan cassava mosaic virus (*SLCMV*)	Mittal *et al*. (2008)
	Curtovirus	*Beet curly top virus* (*BCTV*)	Lee *et al*. (1994)
		Spinach curly top virus (*SCTV*)	Baliji *et al*. (2007)
	Nanovirus	*Faba bean necrotic yellow virus* (*FBNYV*)	Vega-Arreguín *et al*. (2007)
	Mastrevirus	*Bean yellow dwarf virus* (*BeYDV*)	Liu *et al*. (1997)
dsDNA	*Caulimovirus*	***Cauliflower mosaic virus* (*CaMV*)**	Melcher (1989)

Viruses infecting *Arabidopsis thaliana* under natural conditions are indicated in bold.

mosaic virus (TYMV), *Turnip crinkle virus* (TCV) and *Turnip mosaic virus* (TuMV), in six wild *Arabidopsis* populations originating from central Spain during a 4-year period (Pagan *et al*., 2010). Except for TCV and TYMV, which were not detected during the first year of the survey, all viruses were detected every year in at least one *Arabidopsis* population. The reported incidence was maximal for CMV, with an average of 24% of plants infected over all locations and years. The occurrence of co-infection was high, as the percentages of CaMV-, CMV-, TYMV-, TCV- and TuMV-infected plants co-infected with another virus reached 58.2%, 32.9%, 64.7%, 76% and 69.2%, respectively. The fact that all these viruses infect *Brassicaceae* species in their natural habitats suggests that other cruciferous-adapted viruses such as TMV, *Beet western yellow virus* and *Tobacco rattle virus* could also be natural pathogens of *Arabidopsis*.

By contrast, *Arabidopsis* has been reported to be non-host for a number of viruses including several members of the genus *Begomovirus* (*e.g. Squash leaf curl virus*, *Tomato chlorotic mottle virus* and *Tomato golden mosaic virus*) as well as some potexviruses [e.g. *Potato virus X* (PVX) and *Bamboo mosaic virus*] (Hill *et al*., 1998; Jaubert *et al*., 2011; Lin *et al*., 2010; Ribeiro *et al*., 2007; Stenger *et al*., 1992). However, most of these susceptibility analyses were performed using a single *Arabidopsis* accession. For PVX, the lack of infection was shown to involve the antiviral RNA silencing response (Jaubert *et al*., 2011). Indeed, *Arabidopsis* mutants with defects in the essential RNA silencing components, Dicer-like and Argonaute, displayed susceptibility to PVX infection, and PVX was shown to infect plants expressing the viral silencing suppressor of *Pepper ringspot virus*.

Although several studies have demonstrated the impact of viral infections on *Arabidopsis* growth and/or reproduction, an intriguing aspect concerning *Arabidopsis*–virus interactions is that many viruses are asymptomatic or cause only mild symptoms. This feature is particularly obvious when assessing a wide panel of *Arabidopsis* accessions for virus susceptibility. Extensive phenotypic screening with members from the genus *Bromovirus* showed that 59 of 63 *Arabidopsis* accessions infected with *Spring beauty latent virus* and the whole set of accessions infected with *Melandrium yellow fleck virus* displayed no or only mild symptoms, despite accumulating high viral titres (Fujisaki *et al*., 2004; Narabayashi *et al*., 2009). Similarly, no visible symptoms were observed for 28 and 39 *Arabidopsis* accessions susceptible to systemic infection by *Lettuce mosaic virus* (LMV) and *Alfalfa mosaic virus*, respectively (Balasubramaniam *et al*., 2006; Revers *et al*., 2003). This feature could be related to recent insights obtained from viral metagenomic studies, showing that many plant viruses do not cause any obvious symptoms in wild plant hosts (Roossinck, 2012). The frequency of asymptomatic infections may also be overrepresented in *Arabidopsis* because of its status as a model plant species that has led to inoculation assays by many plant viruses.

NATURAL VIRUS RESISTANCE GENES IN *ARABIDOPSIS*

The screening of *Arabidopsis* accessions for their responses to viral infections has led to the identification of resistance genes against viruses belonging to nine genera (Table 2). The large majority of these resistance genes are effective against RNA viruses and many of them have been identified in *Columbia* (Col), which is the most commonly used accession. Similar to observations in crop species, virus resistance genes in *Arabidopsis* control an important diversity of resistance phenotypes ranging from complete resistance, which suppresses virus accumulation either locally (e.g. inhibition of virus replication or cell-to-cell propagation at the primary infection site) or systemically (e.g. inhibition of long-distance movement), to partial resistance associated with reduced and/or delayed virus accumulation and/or with reduced symptom severity. The prevalence of monogenic resistance and the high proportion of recessive resistance genes, approximately 40% of the reported genes, are further common features linking natural virus resistances in *Arabidopsis* and crops (Caranta and Dogimont, 2008). However, besides these phenotypic and genetic similarities, the molecular and functional characterization of natural virus resistance genes in *Arabidopsis* has led to the discovery of novel classes of host genes involved in plant–virus interactions.

The RTM [restricted *Tobacco etch virus* (TEV) movement] resistance system: an original mechanism restricting long-distance movement of potyviruses

The RTM resistance system restricts long-distance movement of viruses from the genus *Potyvirus*, including TEV, *Plum pox virus* (PPV) and LMV (Decroocq *et al*., 2006; Mahajan *et al*., 1998). Three dominant resistance genes, *RTM1*, *RTM2* and *RTM3*, were identified through the analysis of natural genetic variation and ethyl methanesulfonate- or fast neutron-induced mutations in the Col-0 accession (Mahajan *et al*., 1998; Whitham *et al*., 1999). These genes were isolated by a positional cloning strategy. *RTM1* encodes a lectin belonging to a large family of sugar-binding proteins, some members of which are involved in defence mechanisms against a range of bacterial, fungal and insect pathogens (Chisholm *et al*., 2000). *RTM2* encodes a protein with an N-terminal region similar to small plant heat shock proteins, a class of stress-related proteins that play a role in plant defence responses to both viral and nonviral pathogens (Lu *et al*., 2003; Maimbo *et al*., 2007; Whitham *et al*., 2000, 2006). Finally, *RTM3* encodes a MATH (meprin and TRAF homology) protein whose C-terminal end has a coiled-coil domain commonly found in *R*-gene class resistance factors (Cosson *et al*., 2010a). A mutation in any of these genes is sufficient to completely abolish the restriction of the long-distance movement of potyviruses, indicating that they act in an interdependent manner to confer resistance

Table 2 Natural virus resistance genes identified in *Arabidopsis thaliana*.

Virus (genus)	Strain(s)	Accession	Resistance phenotype	Gene(s)	Reference
RNA viruses					
SBLV (*Bromovirus*)	PV-369	Col	Tolerance	*ssb1*	Fujisaki *et al*. (2004)
TCV (*Carmovirus*)	M	Di-0	No systemic movement	HRT+rrt	Cooley *et al*. (2000); Kachroo *et al*. (2000)
CMV (*Cucumovirus*)	Y	C24	No systemic movement	***RCY1***	Takahashi *et al*. (2002)
TRSV (*Nepovirus*)	Grape	Col	Tolerance	***TTR1***	Lee *et al*. (1996)
LMV (*Potyvirus*)	AF199, 0, E	Cvi	No replication or no cell-to-cell movement	*rlm1*	Revers *et al*. (2003)
	0	Col	No replication or no cell-to-cell movement	*LLM1*	Revers *et al*. (2003)
	AF199	Col, Jea, N13, Ws-2, Stw-0, Ita-0, Kn-0	No systemic movement	RTM1+RTM2+RTM3+RTM4+RTM5	Cosson *et al*. (2012); Revers *et al*. (2003)
PPV (*Potyvirus*)	PPV-EA, PPV-PSes, PPV-SK68	Col	No systemic movement	RTM1+RTM2+RTM3+RTM4+RTM5	Decroocq *et al*. (2006, 2009)
	PPV-R	Col	Tolerance and reduced infection	Polygenic (several QTLs)	Decroocq *et al*., 2006; Sicard *et al*., 2008
	PPV-PS	Cvi	No systemic movement	*rpv1*	Decroocq *et al*. (2006)
	PPV-R	Cvi	Intermediate susceptibility	rpv1+rpv3+several QTLs	Sicard *et al*. (2008)
	PPV-R	St-0, RRS-7, Ts-1, Hi-0, Sf-2	No systemic movement	sha3[†]	Pagny *et al*. (2012)
TEV (*Potyvirus*)	HAT, Madison, ST1	Col	No systemic movement	RTM1+RTM2+RTM3+RTM4+RTM5	Chisholm *et al*. (2000); Cosson *et al*. (2010a); Cosson *et al*. (2012); Whitham *et al*. (2000)
TuMV (*Potyvirus*)	Azu, TuR1	Ler	Vascular restriction	*TuNI*	Kaneko *et al*. (2004)
PlAMV (*Potexvirus*)	–	Bay-0	Reduced replication, no systemic movement	***JAX1***	Yamaji *et al*. (2012)
TMV (*Tobamovirus*)	U1	Tsu-1	Delayed and reduced infection	Monogenic, recessive	Dardick *et al*. (2000)
		Col	Delayed infection	*dstm1*	Serrano *et al*. (2008)
DNA viruses					
BCTV (*Curtovirus*)	Logan	Ms-0	No virus movement	Monogenic, recessive	Lee *et al*. (1994)
		Pr-0	No virus movement	Monogenic, recessive	Lee *et al*. (1994)
	CFH	Cen-O	Tolerance	Monogenic, recessive	Park *et al*. (2002)
CaMV (*Caulimovirus*)	CM4-184, CM1841, W260	En-2	Reduced infection	*CAR1*	Callaway *et al*. (1996)

[†]sha3 was identified as a major effect resistance quantitative trait locus (QTL). One to two additional minor effect QTLs contributing to the resistance, sha1 and sha5, have been identified in St-0, RRS-7 and Ts-1.
The cloned genes are in bold and underlined.

BCTV, *Beet curly top virus*; CaMV, *Cauliflower mosaic virus*; CMV, *Cucumber mosaic virus*; LMV, *Lettuce mosaic virus*; PlAMV, *Plantago asiatica mosaic virus*; PPV, *Plum pox virus*; SBLV, *Spring beauty latent virus*; TCV, *Turnip crinkle virus*; TEV, *Tobacco etch virus*; TMV, *Tobacco mosaic virus*; TRSV, *Tobacco ringspot virus*; TuMV, *Turnip mosaic virus*.

(Decroocq *et al*., 2006; Whitham *et al*., 1999). The characterization of the natural diversity of the *RTM* genes from a set of 31 *Arabidopsis* accessions in relation to their ability to restrict the long-distance movement of LMV showed that 40% of the LMV-resistant accessions are controlled by the *RTM* genes (Cosson *et al*., 2012). Allelism tests demonstrated that the LMV susceptibility phenotype is caused by the nonfunctionality of at least one RTM protein, similarly to previous studies showing that the recessive susceptibility alleles contain deletions or nucleotide substitutions resulting in alterations in the amino acid sequence or leading to the introduction of premature stop codons (Chisholm *et al*., 2000; Cosson *et al*., 2010b, 2012; Whitham *et al*., 2000). Interestingly, the Nd-1 accession, for which the three *RTM* genes appear to be functional, was susceptible to LMV, suggesting that additional factor(s) compromise the resistance expected to be conferred by the presence of functional *RTM1*, *RTM2* and *RTM3* alleles. Subsequent genetic analysis identified two new *RTM* loci located on chromosomes 1 and 2, respectively (Cosson *et al*., 2012). In conclusion, the RTM resistance system is widespread among *Arabidopsis* accessions, confers broad-spectrum protection against potyviruses and is conditioned by at least five major dominant genes.

The mechanism underlying the RTM resistance system has yet to be elucidated. It has been shown that the *RTM1* and *RTM2* regulatory sequences are primarily functional in the phloem and that the corresponding proteins localize in vascular-associated tissues (Chisholm *et al*., 2001). These findings, together with data obtained from bimolecular fluorescence complementation experiments that demonstrate direct interaction between the RTM1 and RTM3 proteins (Cosson *et al*., 2010a), suggest that the components of the RTM system may form a multi-subunit complex functioning within the plant vascular system to restrict virus long-distance movement. The fact that the RTM factors function together to confer resistance supports this idea. Further evidence comes from the fact that the N-terminal region of the viral coat protein (CP), which is involved in the long-distance movement of potyviruses, has been mapped as the determinant involved in overcoming RTM-mediated resistance against LMV and PPV (Decroocq *et al*., 2009; Revers *et al*., 1999). In agreement with current knowledge on dominant virus resistance genes (*R* genes), the RTM resistance system could be part of an active plant defence mechanism. In addition to the occurrence of many RTM protein domains involved in protein–protein interactions, RTM proteins also share features with proteins involved in the plant defence response. Plant lectins with similarities to RTM1 are involved in defence against many plant pathogens, including several fungi and insects (Vandenborre *et al*., 2011). Proteins with an hsp (heat shock protein) domain, such as RTM2, have been characterized as a class of stress-related proteins that play a significant role in plant defence responses to both viral and nonviral pathogens (Lu *et al*., 2003; Maimbo *et al*., 2007; Whitham *et al*., 2000, 2006). RTM3 harbours a coiled-coil domain commonly found in *R*-gene class resistance factors. This domain is required for the interaction of RTM3 with RTM1 (Cosson *et al*., 2010a), and its mutation has been shown to impair RTM-mediated resistance (Cosson *et al*., 2012). An additional argument for the involvement of RTM resistance in an antiviral defence response with similarities to *R* genes was the observation that the expression of the three cloned *RTM* genes is modified by hormonal stimuli, independently of viral infection (Cosson *et al*., 2012). However, in comparison to classical *R*-gene-mediated resistance, the RTM resistance system differs in that it is not race specific and does not involve typical hallmarks such as a hypersensitivity response (HR) (i.e. localized cell death reactions confining the virus to initially infected cells), pathogenesis-related (*PR*) gene expression or salicylic acid (SA)-dependent defence signalling (Decroocq *et al*., 2006; Mahajan *et al*., 1998; Revers *et al*., 2003). RTM-mediated resistance also appears to be independent of RNA silencing, as mutations of factors required for this antiviral defence mechanism do not compromise resistance to potyviruses (Cosson *et al*., 2010b).

Altogether, these results indicate that RTM resistance may represent a novel form of plant antiviral mechanism. Further understanding of this resistance pathway, which to date has only been described in *Arabidopsis*, will not only uncover new mechanisms underlying the resistance strategies adopted by plants to combat potyvirus infection, but will also shed light on the molecular events associated with the long-distance movement of these viruses.

A significant role for lectins in *Arabidopsis*–virus interactions

Exciting insights into dominant resistance to plant viruses have been provided by the recent cloning and functional characterization of the *JAX1* (*JACALIN-TYPE LECTIN REQUIRED FOR POTEXVIRUS RESISTANCE 1*) gene, which confers resistance to potexviruses (Yamaji *et al*., 2012). JAX1-mediated resistance was identified in the *Arabidopsis* accession *Bayreuth-0*, where it suppresses the accumulation of *Plantago asiatica mosaic virus* in the inoculated leaves. Heterologous expression of *JAX1* in *Nicotiana benthamiana* demonstrated that it confers cellular-level resistance to several other members of the genus *Potexvirus*, including PVX, *White clover mosaic virus* and *Asparagus virus*, whereas it has no effect on infection by viruses from other genera, including *Como-*, *Cucumo-*, *Poty-*, *Tobamo-* and *Tobravirus*. Subsequent map-based cloning revealed that *JAX1* encodes a new member of the lectin protein family, similar to the previously characterized *RTM1* resistance gene involved in the inhibition of the systemic movement of potyviruses. These data suggest that lectins play a significant role in *Arabidopsis*–virus interactions.

Lectins are known to act as pathogen recognition molecules involved in innate immune defense mechanisms in both vertebrates and invertebrates (Vasta *et al*., 2007). In plants, several

lectins have been reported to show inhibitory effects against bacteria, fungi or insects, supporting an evolutionary conserved function in defence mechanisms (Peumans and Van Damme, 1995; Van Damme *et al*., 2004). *In vitro* studies have demonstrated that plant lectins inhibit the accumulation of various mammalian viruses, probably through their binding to glycosylated viral proteins (Balzarini *et al*., 2005; Lam and Ng, 2011). These features led to the hypothesis that RTM- and JAX1-mediated resistance could be induced by the recognition of glycosylated viral proteins, resulting in the inhibition of viral accumulation (Yamaji *et al*., 2012). Both the RTM1 and JAX1 proteins contain a conserved sugar-binding domain. The N-terminal region of the viral CP, which is involved in overcoming RTM-mediated resistance, is glycosylated in both poty- and potexviruses (Baratova *et al*., 2004; Decroocq *et al*., 2009; Fernandez-Fernandez *et al*., 2002). The role of post-translational modifications of the CP, including *O*-glycosylation as a parameter influencing the outcome of RTM-mediated resistance was investigated (Decroocq *et al*., 2009). Computer predictions did not show a significant difference in total phosphorylation or glycosylation residues between RTM-breaking and RTM-restricted PPV isolates, and infection of *sec-2* (for *secret agent-2*) or *spy-1* mutants [*O*-linked *N*-acetylglucosamine transferase (OGT)-depleted mutants] showed that reduction of the activity of one or the other *Arabidopsis* OGTs did not alleviate resistance. These results do not support the involvement of *O*-glycosylation in RTM-mediated resistance.

Similarly to RTM1, JAX1-triggered resistance is independent of cell death reactions (HR), *PR* gene expression, hormone signalling and RNA silencing. Some specificity also exists. Whereas *RTM1* is exclusively expressed in vascular tissues, *JAX1* is expressed in both vascular and mesophyll cells. Moreover, unlike RTM1, JAX1 does not require additional factors to restrict viral infection. It has been proposed that these distinct features might reflect a role for lectins in controlling different resistance levels targeted against viruses belonging to distinct viral genera (Yamaji *et al*., 2012). In these respects, lectin-mediated resistance is reminiscent of *R*-gene-mediated resistance, which is characterized by narrow recognition specificity and associated with either cellular-level or systemic-level resistance. Collectively, these data lead to the challenging idea that lectins could play an important role in dominant resistance mechanisms that may be viewed as a new layer of plant immunity against viral infection processes.

Dominant resistances mediated by the nucleotide-binding, leucine-rich repeat (NB-LRR) family

Two dominant resistance genes have been cloned in *Arabidopsis*, *HRT* (*HYPERSENSITIVE RESPONSE TO TCV*) and *RCY1* [*RESISTANCE TO CUCUMBER mosaic virus (Y)*], which belong to the well-known NB-LRR family of resistance (*R*) genes, as do all other known dominant virus resistance genes cloned in crops (recently reviewed by Cournoyer and Dineskumar, 2011). *HRT* and *RCY1* correspond to two alleles at the same locus identified in the Di-0 and C24 accessions, respectively (Cooley *et al*., 2000; Takahashi *et al*., 2002). Although the HRT and RCY1 proteins show high identity at the amino acid level (91.3%), they specifically control only their cognate viral pathogens through distinct defence signalling pathways. HRT confers systemic resistance to TCV in an SA-dependent manner (Kachroo *et al*., 2000). By contrast, RCY1 confers systemic resistance to CMV, is only partially dependent on SA and involves ethylene signalling (Takahashi *et al*., 2002). Another intriguing aspect that distinguishes HRT- from RCY1-mediated resistance, and makes HRT an atypical resistance system, is the fact that *HRT* requires a recessive gene of unknown function, named *rrt* (*regulates resistance to TCV*), to induce efficient resistance against TCV. Genetic analysis of the inheritance of TCV resistance demonstrated that *HRT* is sufficient to induce typical hallmarks of *R*-gene-triggered resistance, including HR formation and activation of *PR* gene expression, but requires the function of *rrt* to restrict viral accumulation (Kachroo *et al*., 2000). Interestingly, it was shown that the requirement of *rrt* for resistance to TCV could be overcome by up regulating the expression of *HRT*. Transgenic plants expressing *HRT* at very high levels are resistant to TCV, even in an *RRT* background (Cooley *et al*., 2000). Similarly, SA was shown to confer TCV resistance in *RRT*-containing plants by increasing *HRT* transcripts (Chandra-Shekara *et al*., 2004). Based on these observations, it has been proposed that RRT suppresses resistance and that high levels of *HRT* expression overcome this effect, thereby suggesting that *rrt* might correspond to a nonfunctional version of a dominant negative defence regulator (Chandra-Shekara *et al*., 2004).

A study to characterize host factors regulating symptom expression during infection by *Tobacco ringspot virus* (TRSV) has led to the identification of a third NB-LRR gene involved in *Arabidopsis*–virus interactions (Lee *et al*., 1996). Most *Arabidopsis* accessions display tolerance to TRSV. The Col-0 and *Estland* accessions were shown to accumulate TRSV to similar levels, but Col-0 plants remained symptomless whereas *Estland* plants developed lethal systemic necrosis. Genetic and functional analyses have demonstrated that a single locus, designated *TTR1* (TOLERANCE TO TRSV 1), controls TRSV tolerance versus lethal systemic necrosis, and that the TTR1-induced necrotic phenotype is dependent on SA signalling (Nam *et al*., 2011). Site-directed mutagenesis identified two critical amino acid residues in the TTR1 protein involved in the elicitation of the necrosis response. Therefore, *TTR1* resembles other genes that confer resistance, but, rather than making plants resistant to infection, the gene, with characterized mutations, induces a misdirected plant defence response that kills the plant. Similar findings have been reported by Kim *et al*. (2008). Upon infection with TuMV, the *Landsberg erecta* accession was found to develop a vascular necrosis that spreads systemically and results in plant death. This response resembles an HR-like cell death

reaction and is associated with an increased production of both SA and ethylene and the expression of several defence-related *PR* genes. The TuMV-induced necrotic phenotype is controlled by a dominant locus, named *TuNI* (*TuMV NECROSIS INDUCER*), which co-localizes with an NB-LRR-encoding gene on chromosome 1 (Kaneko *et al*., 2004). These two examples illustrate the narrow border between resistance and susceptibility.

Arabidopsis represents a source of new recessive virus resistance genes

Several naturally occurring recessive resistance genes against viruses have been identified in *Arabidopsis*, but none has yet been cloned (Table 2). Currently, the elucidation of the molecular nature of this class of resistance genes has exclusively been reported in crops, and has so far only revealed a group of proteins linked to the translation machinery, chiefly the eukaryotic translation initiation factors (eIFs) 4E and 4G (recently reviewed by Le Gall *et al*., 2011). Many results obtained argue in favour of a resistance mechanism mediated by subtle amino acid change(s) in the protein encoded by the recessive resistance alleles, which impair the interaction of translation initiation factors with viral proteins, thereby leading to the inability of the virus to successfully infect the plant. These mutant alleles control resistance against a wide array of RNA viruses, and have been identified in a variety of crop species, including the dicots lettuce (*mo1*), melon (*nsv*), pea (*sbm1*), pepper (*pvr1/2/6*) and tomato (*pot1*), and the monocots barley (*rym4/5*) and rice (*rymv1*).

A striking feature of *Arabidopsis*, in comparison with crops, is that although infectivity assays on T-DNA mutants or protein–protein interaction studies have converged towards the identification of eIF4E and eIF4G as key players in *Arabidopsis*–RNA virus interactions (Le Gall *et al*., 2011), eIF4-mediated resistance has never been identified in the natural diversity of this species. The recessive resistance genes *rlm1*, for resistance to LMV, and *rpv1*, for resistance to PPV, both identified in the *Cape Verde Islands* (Cvi) accession, do not implicate translation initiation factors because they were mapped to genomic regions containing no *eIF* genes (Decroocq *et al*., 2006; Revers *et al*., 2003). The lack of co-segregation with *eIF4E* or *eIF4G* genes also holds true for *dstm1*, which is responsible for the delayed systemic movement of TMV in the Col accession (Serrano *et al*., 2008), and for *sha3*, a major quantitative trait locus (QTL) contributing to systemic resistance against PPV in several *Arabidopsis* accessions (Pagny *et al*., 2012). This feature is also supported by the genetic diversity analysis at the *eIF4E* and *eIF4G* loci (Charron, 2007). The systematic sequencing of *eIF4E* and *eIF4G* genes in a core collection of 54 accessions capturing more than 90% of the genetic diversity in *Arabidopsis* failed to identify signature amino acid substitutions previously demonstrated to be responsible for eIF4E/4G-mediated virus resistance in crops. These data indicate that the molecular cloning of these resistance genes will lead to the characterization of new host factors required for viral life cycles. This viewpoint can be exemplified by the results obtained from the characterization of *dstm1*. Electron microscopy analysis revealed the accumulation of defectively assembled virions in the vascular tissues of the petioles of inoculated leaves and stems of Col plants, suggesting that *dstm1* may encode a host factor participating in the stability or correct assembly of virus particles in the vascular system. In line with this idea, preliminary mapping data localized *dstm1* to a genomic region containing several genes related to transport function or encoding cell wall enzymes involved in the systemic movement of TMV (Serrano *et al*., 2008). Another promising example concerns *sha3*. Classical linkage mapping combined with quantitative genome-wide association mapping delimited this resistance locus into a genomic region containing a MATH-related gene cluster, thereby raising the possibility that MATH proteins might control the restriction of PPV systemic infection (Pagny *et al*., 2012).

MODULATION OF *ARABIDOPSIS*–VIRUS INTERACTIONS BY THE ENVIRONMENT

Our current understanding of the mechanisms associated with virus resistance in *Arabidopsis* has almost exclusively come from studies conducted under controlled conditions, while environmental factors are important determinants shaping host–pathogen interactions. Numerous studies have examined the effects of environmental factors on host responses to bacterial, insect or fungal pathogens (Murdock *et al*., 2012; Roden and Ingle, 2009), but research in this field has been hitherto rather limited for plant viruses.

A few studies conducted in *Arabidopsis* have shown that the environment strongly modulates symptomatic versus asymptomatic viral infections. For example, field-grown *Arabidopsis* plants infected with CaMV, CMV, TYMV, TCV or TuMV were found to display no obvious symptoms despite accumulating high viral titres, whereas all these viruses cause severe symptoms after infection under laboratory conditions (Pagan *et al*., 2010). Although recent insights obtained from viral metagenomics indicate that natural symptomless infections of wild species appear to be a general rule rather than an exception (Roossinck, 2012), such data underline the importance of the environment in the expression of symptoms and probably in the outcome of infection. Virus symptom development in *Arabidopsis* may also differ under different laboratory conditions. Such situation is exemplified by the finding that plants infected with CMV, CaMV or *Turnip vein clearing virus*, and grown under continuous light conditions, display enhanced chlorotic and leaf distortion symptoms compared to plants infected with the same viruses, but grown under diurnal light conditions (Handford and Carr, 2007). The consequence of the absence of a diurnal light regime on symptom intensity was

related to starch metabolism and supports the existence of sugar-mediated control of viral symptom development. For CaMV, it has also been shown that infected plants maintained under short days develop much more severe symptoms than plants grown under long days (Cecchini *et al*., 1998). Interestingly, phenotypic analysis of late-flowering mutants of *Arabidopsis* demonstrated that the underlying mechanism is related to the vegetative versus reproductive plant stage, whereby the onset of flowering negatively affects symptom development (Cecchini *et al*., 2002).

Environmental conditions also modulate resistance phenotypes and mechanisms, particularly those associated with plant defence responses. In the *Arabidopsis*–TuMV interaction, the *TuNI*-induced vascular necrotic phenotype has been shown to be regulated in a light-dependent manner. A shading treatment of 24 h prior to TuMV inoculation impaired the HR-like programmed cell death, and caused a significant decrease in the levels of *PR-1* and *PR-5* gene expression as well as reduced production of SA (Kim *et al*., 2008). Light requirement for the induction of the plant defence response has also been reported in the HRT/*rrt*-triggered resistance of *Arabidopsis* to TCV. In this case, light appeared to be important for resistance during the first hours following TCV inoculation. Plants subjected to 48 or 72 h of darkness immediately after TCV inoculation exhibited a marked decline in *PR-1* transcript levels, reduced HR formation on inoculated leaves and enhanced susceptibility to TCV infection (Chandra-Shekara *et al*., 2006). Although the lack of light did not affect TCV-induced SA production, it was demonstrated that treatment with exogenous SA prior to TCV inoculation increased resistance in plants that were shifted to darkness after inoculation, suggesting that light might be required to trigger SA-mediated signalling (Chandra-Shekara *et al*., 2006).

More recently, Jeong *et al*. (2010) showed a direct role for blue-light photoreceptors in regulating light-dependent HRT-mediated resistance to TCV. In initial experiments, dark-infected Di-17 plants expressing an epitope-tagged HRT protein (HRT-FLAG) were found to accumulate reduced levels of HRT-FLAG compared with light-infected plants, suggesting that the dark-conferred susceptibility to TCV in *Arabidopsis* was associated with a degradation of the HRT resistance protein. This dark-triggered degradation of HRT was then shown to reflect impairment of the blue-light photoreceptors cryptochrome 2 (CRY2) and phototropin 2 (PHOT2). Mutations in either of these genes conferred susceptibility to TCV and triggered reduced stability of the ectopically expressed HRT-FLAG protein. In addition, TCV susceptibility and HRT-FLAG degradation were observed in HRT-FLAG wild-type plants subjected to blue light, which is known to cause degradation of CRY2. Taken together, these results suggest that a blue-light photoreceptor-mediated pathway is required for the post-transcriptional stability of HRT and, consequently, for resistance to TCV. In further experiments, the HRT protein was shown to interact with the CRY2/PHOT2-interacting protein COP1, an E3 ubiquitin ligase involved in 26S proteasome-mediated protein degradation, whose activity is likely to be repressed by CRY2 and PHOT2 (Mao *et al*., 2005; Wang *et al*., 2001). Although the degradation of HRT via the CRY2/PHOT2-regulated COP1 protein was not demonstrated, the finding that pretreatment of HRT-FLAG-expressing plants with a 26S proteasome-specific inhibitor significantly inhibited the blue-light-triggered degradation of HRT-FLAG and conferred resistance to TCV infection strongly suggests that the CRY2 and/or PHOT2 photoreceptors, probably in complex with COP1, regulate HRT/*rrt*-mediated resistance to TCV by preventing proteasome-dependent degradation of the HRT resistance factor. In light of these findings, consideration of environmental factors is an important step towards understanding the principles underlying both viral pathogenesis and plant resistance mechanisms.

CONCLUSIONS AND FUTURE CHALLENGES

Natural virus resistance has been thoroughly studied in *Arabidopsis* and has provided original insights into the genes and mechanisms by which plants combat these pathogens. Thus far, seven virus resistance genes identified in diverse *Arabidopsis* accessions have been characterized at the molecular level using positional cloning approaches. Among these genes, four were shown to control resistance mechanisms that have not yet been discovered in crop plants. Several recessive resistance genes were also identified that do not correspond to the widespread eIF4-mediated resistance mechanism. Taken together, these data point towards a distinct mode of evolution of virus resistance in *Arabidopsis* and in crops. A hypothesis to explain this feature is that *Arabidopsis* and crops may have evolved different ways to counteract viral attacks. An assessment of the extent to which experimental *Arabidopsis*–virus pairs also occur in natural conditions should be considered in order to provide a more comprehensive view of the evolutionary interplay between *Arabidopsis* and viruses. Addressing this issue should be facilitated by recent developments in plant virus metagenomics which permit the study of viruses in environmental samples using next-generation sequencing (for a recent review, see Roossinck, 2012). Furthermore, as pointed out in this article, we must assume that the features of *Arabidopsis*–virus interactions and resistance mechanisms determined under controlled laboratory conditions probably differ from those that would be found under fluctuating natural conditions. A more extensive analysis and a better picture of the importance of environmental influences on *Arabidopsis*–virus systems are therefore needed. We believe that these issues are worth pursuing in future research programmes because they may provide data needed to more accurately exploit such virus resistance mechanisms in crops.

At the same time, *Arabidopsis* research has produced convincing evidence that the elucidation of the mechanisms at the interfaces between environment, plant development and responses to

viral infections will lead to improve our knowledge on the principles underlying both viral pathogenesis and plant resistance mechanisms. It is also becoming increasingly evident that *Arabidopsis* represents an ideal system to shed light on novel genes and mechanisms mediating resistance against viruses. Beside natural resistance factors, host factors interacting with viral proteins, RNA or DNA, or directly involved in a specific viral cycle step, are also promising candidates for new resistance sources in crops (for a recent review, see Huang *et al*., 2012). However, the translational potential of this knowledge in optimized genetic strategies to tackle virus disease problems in crops is an issue that still needs to be addressed. For such a challenge, genetic tools are available. For example, TILLING (Targeting Induced Local Lesions in Genomes) offers a unique opportunity for the rapid and reliable identification of new alleles in genes of particular interest (recently reviewed by Kurowska *et al*., 2011). In addition, recent advances in next-generation sequencing and bioinformatics tools to identify homologous counterparts of *Arabidopsis* resistance genes will allow large-scale and cost-effective sequencing of available crop germplasm collections to search for naturally occurring virus resistance-associated mutations in predefined genes of interest. Although this remains to be precisely determined, we can expect that the translational potential will probably be higher for simple recessive resistance systems corresponding to mutations in host factors required for the viral infectious cycle, involving genes from small multigenic families and for which mutations do not affect plant fitness, than for complex resistance systems, such as RTM, involving several genes from large gene families.

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