# Mechanisms of Resistance to Viruses

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**Abstract**

Resistance associated with a hypersensitive response (HR) and subsequent development of necrotic lesions (cell death) at the sites of virus infection can restrict virus movement in plants. Genes for HR are dominant and act on a gene-for-gene basis. Many viral proteins triggering HR have been identified. Also, several genes for HR-based virus resistance, or virus-induced cell death without resistance, have been isolated and characterized in plants, which provides novel insights to the mechanisms of virus resistance. Another international, major research frontier has formed more recently around RNA silencing, a universal RNA surveillance system and inducible virus defence mechanism in multicellular organisms. Many viral proteins interfere with different phases of RNA silencing. The data provide novel insights to break-down of resistance in mixed virus infections (viral synergism), resistance to virus movement, and recovery of plants from virus infection.

**Keywords:** virus resistance; gene-for-gene interaction; RNA silencing

## INTRODUCTION

The dominant resistance (*R*) genes operating on a gene-for-gene basis (FLOR 1946) are required for pathogen recognition and initiation of signal transduction, which leads to activation of the defense genes conditioning the cells and tissues to resist invasion of the pathogen (ELLIS *et al*. 2000; YOUNG 2000). The *R* gene product interacts specifically (directly or indirectly) with a product produced by action of a pathogen avirulence gene (*Avr*) (FREDERICK *et al*. 1998; ELLIS *et al*. 2000; WHITE *et al*. 2000; YOUNG 2000).

The two main types of *R* gene-mediated resistance to viruses are extreme resistance (ER) and hypersensitive resistance (HR). ER strongly suppresses virus accumulation in infected cells. No visible symptoms and no detectable amounts of virus are observed in inoculated plants. In contrast to ER, HR is usually strain-specific and characterized by development of necrotic lesions (cell death) at the initial infection sites on inoculated leaves. HR does not restrict virus accumulation in infected cells but it may prevent the spread of virus within and from the inoculated leaf. Isolation, transformation to the same host, and subsequent comparison of the resistance responses triggered by the ER (*Rx*) and HR (*N*) genes indicates that the genes for ER induce resistance earlier than the HR genes (BENDAHMANE *et al*. 1999). Therefore, the strong resistance against virus multiplication conferred by the genes for ER (BARKER & HARRISON 1984; KÖHM *et al*. 1993) probably prevents accumulation of virus to the concentrations required for activation of HR.

The actual resistance mechanisms for ER and HR are unknown. In some cases, HR may be activated but fails to restrict virus movement in plant tissues, which results in the development of larger necrotic lesions, vein necrosis or lethal necrosis in the inoculated leaf and, also, in other parts of the plant following systemic virus movement. Higher temperatures can modulate expression of HR, reduce its efficiency to restrict viral movement and cause extensive tissue necrotization symptoms. A well-studied example is the temperature-sensitive expression of HR to *Tobacco*

*mosaic virus* (TMV, genus *Tobamovirus*) conferred by the *N* gene in tobacco (*Nicotiana tabacum*). At 22°C, HR is activated upon infection with TMV, necrotic local lesions develop and no systemic infection with TMV is observed, whereas at 32°C no HR is observed and plants are systemically infected with mosaic symptoms (ROSS 1961; WESTEIJN 1981; DINESH-KUMAR & BAKER 2000).

Six genes recognizing viruses on the gene-for-gene basis have been cloned and described from plants: *N* from tobacco (WHITHAM *et al*. 1994), two nearly identical *Rx* genes conferring ER to *Potato virus X* (PVX, genus *Potexvirus*) in potato (BENDAHMANE *et al*. 1999, 2000), *Sw-5* conferring resistance against systemic infection with tospoviruses in tomato (BROMMONSCHENKEL *et al*. 2000), *HRT* conferring HR to *Turnip crinkle virus* (genus *Carmovirus*) in *Arabidopsis* (COOLEY *et al*. 2000), and *Y-1* inducing cell death upon infection with *Potato virus Y* (genus *Potyvirus*) in potato (VIDAL *et al*. 2002). These genes share a high degree of structural similarity with each other, but also with other pathogen-specific *R* genes (ELLIS *et al*. 2000; VIDAL *et al*. 2002). Most of them belong to the LZ-NBS-LRR class (leucine zipper – nucleotide binding site – leucine rich repeat) of *R* genes, but *N* and *Y-1* belong to the TIR-NBS-LRR class (TIR for '*Toll* and interleukine receptor-like'; WHITHAM *et al*. 1994; VIDAL *et al*. 2002). In contrast to the structural similarity shared by the *R* genes, the viral avirulence proteins eliciting the *R*-gene mediated resistance do not share any significant similarity (WHITE *et al*. 2000; VALKONEN 2002).

Plants also express recessive genes for resistance to viruses, mainly for restricting viral cell-to-cell or long distance movement but sometimes also virus multiplication or accumulation in infected cells (NICOLAS *et al*. 1997; KELLER *et al*. 1998; HÄMÄLÄINEN *et al*. 2000).

RNA silencing is a natural defense mechanism against viruses in multicellular eukaryotic organisms (reviewed by CHICAS & MACINO 2001; BAULCOMBE 2002). It results in rapid and specific degradation of cytoplasmic RNAs and accumulation of small 21–25 nucleotides-long RNA fragments (small interfering RNAs; siRNAs) originating from the target sequence, which is diagnostic of RNA silencing (HAMILTON & BAULCOMBE 1999). The most potent inducer of RNA silencing is double-stranded RNA (dsRNA). The onset of RNA silencing is followed by a propagation phase, and delivery of a systemic signal to other parts of the plant where homologous RNA molecules will be silenced. The nature of the signal is yet unknown, but the signaling pathway follows the transport of macromolecules and viruses through plasmodesmata between cells and via phloem over long distances (RUIZ-MEDRANO *et al*. 2001).

In some cases, plants are initially susceptible to the virus and develop a systemic viral infection, but resistance is later induced in developing leaves. The recovered leaves are virus free and resistant to new infections with the same virus (COVEY *et al*. 1997; RATCLIFF *et al*. 1997). siRNAs derived from the virus are detected in the recovered leaves. Localized recovery, or dark green islands (DGIs), may develop on chlorotic, virus-infected leaves. No viral nucleic acids and proteins are detected in DIGs, they are resistant to infection with the same virus (MOORE *et al*. 2001), and siRNA specific to the virus accumulate in them (YELINA *et al*. 2002). In tobacco plants, silencing of the gene *NtRDRP1* that encodes an RNA-dependent RNA polymerase (a host factor required for RNA silencing), prevents the formation of DGIs after infection with TMV (XIE *et al*. 2001). A viral RNA suppressor alone reduces DGI formation and is sufficient to prevent systemic recovery of plants from viral infection (YELINA *et al*. 2002).

Plant viruses have evolved to suppress and/or circumvent RNA silencing using some of their proteins (VOINNET *et al*. 1999). Viral silencing suppressors probably have different molecular targets in the host and, thus, interfere with different parts of the silencing pathway (initiation, signaling or maintenance phases). The viral RNA silencing suppressors are determinants of additional phenotypes such as viral vascular transport (BRIGNETI *et al*. 1998). They also determine symptom severity (virulence) as shown by the enhanced virulence of a chimeric PVX that expresses heterologous RNA silencing suppressors derived from a wide range of virus genera (SCHOLTHOF *et al*. 1995; BRIGNETI *et al*. 1998). Furthermore, RNA silencing is involved in cross-protection between viruses in co-infected plants. This can be shown by co-infection of plants with chimeric TMV that expresses GFP and chimeric PVX carrying a truncated *gfp* gene (RATCLIFF *et al*. 1999). The truncated *gfp* is unable to produce functional GFP but is sufficient to trigger RNA silencing, observed as an inability of TMV-GFP to move systemically in co-infected plants. In contrast, expression of a viral RNA silencing suppressor from one of the virus chimeras allows systemic movement of TMV-GFP. This result indicates that cross-protection is a manifestation of RNA silencing, and it also infers that RNA silencing restricts vascular movement of viruses.

It is hypothesized that co-evolution of viruses and their plant hosts has resulted in mechanisms that allow

the host to inhibit, restrict or tolerate viral infections and the viruses to evade host defenses (YELINA *et al.* 2002). The *R* gene-mediated resistance has evolved to inhibit infection with specific viruses, but viruses can overcome it due to mutations (amino acid substitutions) introduced to the viral AVR proteins during virus replication. On the other hand, RNA viruses replicate via dsRNA intermediates that are in general recognized and targeted by the host RNA surveillance system. Consequently, to replicate their genomes, viruses have developed mechanisms to counteract RNA silencing by producing proteins that suppress it. The opposite forces of silencing induction by dsRNA and silencing suppression by the viral RNA suppressors may then determine, whether or not the plants are infected, or recover from infection.

The *R* gene-mediated resistance and RNA silencing both involve systemic signaling, which induces resistance in other parts of the plant. It is interesting to note that the systemic resistance induced by the virus-specific *R* genes is broadly effective against different types of pathogens, whereas local activation of the general dsRNA degradation mechanism (RNA silencing) induces virus-specific systemic resistance. Future studies will show how closely the two inducible resistance mechanisms are linked at the functional level.

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