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LITERATURE REVIEW

Plant-virus interactions during the infective process

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Abstract

C. Stange. 2006. Plant- virus interactions during the infective process. Cien. Inv. Agr. (in English) 33(1):1-18. Viruses that infect plants are generally single-stranded (ss) positive-sense RNA viruses. The accumulation of the virus progeny inside the plant cells involves translation, replication, cell–to-cell and long-distance movement of viral sequences. Over the past 30 years high progress has been made in understanding the interactions between the virus and the host plant during these processes. Reports of host factors implicated in promoting viral cycle and the characterization of plant virus receptors (R) and their resistance mechanisms in Solanaceae, Cucurbitaceae, Leguminoseae and in *Arabidopsis thaliana* have contributed extensively to understanding this complex interaction. Almost all of the R genes cloned share structural similarity, harbouring LRR, NBS, TIR and LZ domains, suggesting a convergence in the signal transduction machinery in plant defence. Plant viruses evolve very rapidly. This is possible because of their very short replication cycles, large numbers of genomes within each cell and across many cells per host, and many hosts infected. Therefore, viruses readily produce new avirulence factors and resistance-breaking viral genotypes. To overcome the appearance of new viral races, plants generate R gene variants through recombination processes and develop specialized defence mechanisms such as post-transcriptional gene silencing. However, viruses such as *Potyvirus X* can overcome this type of plant resistance. Recent insights into virus-host interactions have been compiled in this review, focusing on the interaction between *Tobacco mosaic virus* and the N receptor in *Nicotiana tabacum*, to describe the possible transduction mechanisms that trigger a cascade of downstream events leading to viral defence in plants.

Key words: *N* gene, plant resistance genes, TMV, virus, virus movement, viral replication.

Introduction

Plant viruses are infective particles considered obligate intracellular parasites usually composed of positive single-stranded ribonucleic acid (ssRNA) and only in a few cases by single-stranded or double-stranded deoxyribonucleic acid (ssDNA and dsDNA, respectively). Viruses can only enter the plant cell passively through wounds caused by physical injuries due to environmental factors or by vectors. Among vectors, several species of insects, mites, nematodes and some soil inhabitant fungi can transmit specific viruses. In the cytoplasm, the RNA disassembles, replicates, converts its mRNA to proteins, and mobilizes locally and systemically. Viruses use energy and proteins from the host cell to perform these processes.

Different interactions are generated between the plant and the virus during each stage of the viral cycle. If the viral particle is not recognized by the host plant, a compatible interaction between the plant and the virus is established. This interaction may be favorable for the virus (Hammond-Kosack and Jones, 2000). However, if the plant recognizes the viral particle, an incompatible interaction that is unfavorable for the virus is established. It is known that plants can recognize the virus, limiting it to the site of the infection. A series of complex cascade defence reactions can be induced, limiting virus replication and virus movement within the host

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plant (Hammond-Kosack and Jones, 2000).

Plant-virus interactions are extremely complex and have been studied in depth for more than half a century. As a consequence, the mechanisms linked to viral accumulation inside host cells, movement of virus within the plant as well as the plant defence mechanisms, have been partially elucidated.

The objectives of this review are: 1. To present the latest evidences related to replication, translation, and viral movement within the host plants, 2. To discuss the importance of the host factors associated with these processes, 3. To present and discuss the plant defence mechanisms that enable recognition and subsequent resistance to viral infections, 4. To discuss the currently described virus resistance genes, using as an example the receptor N-*Tobacco mosaic virus* (N–TMV) interaction, and 5. To suggest possible transduction mechanisms which ultimately assist the resistance process.

Viral replication and translation

For DNA and RNA type plant viruses, viral accumulation within plant cells depends on replication and translation processes (Ahlquist *et al*., 2003; Buck, 1999; Hanley-Bowdoin *et al*., 2004; Ishikawa and Okada, 2004; Noueiry and Ahlquist, 2003).

In contrast to messenger RNA (mRNA), viral RNAs may have several structures in the 5' region, such as a phosphate group, a cap, or a polypeptide termed *VPg* (*Viral protein genome-linked*). Some of these structures are very different from the mRNA cap found in the host. Some viruses have *IRES (internal ribosome entry sequence),* which allow translation without needing the initiating *elF4F* complex. On the other hand, viral 3' region may have a *polyA* tail as in an RNA structure, or a free OH group (Thivierge *et al*., 2005).

For viral replication, positive sense ssRNA viruses have RNA dependent RNA polymerases (*RdRp*). Nevertheless, they require certain factors from the host to establish the replication process. Replication process begins by copying the (+) strand into a complementary (-) strand, which is an intermediate that serves for the production of more genomic (+) RNA. These progeny are used as template for translation and replication of (+) strands that will be part of new virions. The translation and replication of the same template is a process in which the ribosomes and *RdRp* activity must be regulated and controlled (Barry and Miller, 2002). Despite the absence of *cap* and *polyA* in its extremes, recent evidence suggests that viral RNA circularizes just like mRNA (Thivierge *et al*., 2005). This enables viruses to have access to the translation machinery of the host, relocate the *RdRp*, and efficiently translate its proteins (Barry and Miller, 2002; Borman *et al*., 2000; Herold and Andino, 2001; Le *et al.*, 1997; Wei *et al*., 1998).

Structural proteins, such as the capsid protein (CP), replicase, movement proteins, and other specific viral proteins are obtained during the translation process. During viral replication, they produce multiple copies of the same viral genome that uses the symplastic pathway to establish a systemic infection in susceptible plants (Lucas *et al*., 1995). In a compatible reaction, the ability of the virus to invade the plant is based on the formation of heterocomplexes between viral and host proteins. The virus uses the symplastic path to establish a systemic infection in susceptible plants (Lucas *et al.*, 1995). In addition, there are other proteic factors in the host like the receptors coded by resistance genes. As discussed later, the presence of these specific resistance genes limits the local and systemic movement of the viral particles during an incompatible interaction.

Virus accumulation and movement

The cell-to-cell movement is an early event in the infective process. It has been described that *Tobacco rattle virus* in *Nicotiana clevelandii* and TMV in *N. tabacum* move from one cell to another in 4 and 5 h, respectively (Derrick *et al*., 1992; Fannin and Shaw, 1987).

During the first stages, virally-encoded movement proteins (MP) bind to the viral genome and transport it from epidermal to mesophyll cells through the plasmodesmata, until it reaches the vascular bundles.

Host factors linked to the viral infective cycle have been identified through mutagenesis. These preferentially act on cell-to-cell and systemic movement. For example, *Arabidopsis* mutants *tom1* and *tom2*, prevent accumulation of TMV in an infected cell. *Tom1* and *Tom2* encode transmembrane tonoplast proteins that mutually interact and bind with the helicase domain of virus replicase.

On the other hand, there is evidence that the cytoskeleton and its components facilitate viral transport through the plasmodesmata. Many viral MPs are localized in the plasmodesmata, arriving via the endoplasmic reticulum (ER). The actin/myosin filaments regulate protein flow through the ER (Boevink and Oparka, 2005; Liu *et al.,* 2005).

Several reports have demonstrated that callose (a ß 1-3 glucan) is deposited in the plasmodesmata during the hypersensitivity reaction (HR), thus blocking these channels and preventing cell-to-cell viral movement (Wolf *et al.*, 1991; Beffa *et al.*, 1996; Iglesias and Meins, 2000; Bucher *et al.*, 2001). TGB2 protein of *Potato virus X* (PVX) interacts with ß-1,3-glucanase, a callose degrading enzyme (Fridborg *et al*., 2003). This leads to accelerated degradation of callose, thus facilitating PVX movement through the plasmodesmata (Fridborg *et al*., 2003).

The Closterovirus have an Hsp70 homolog protein with MP activity and with high affinity for microtubules (Peremyslov *et al*., 1999; Alzhanova *et al*., 2001). Also, it has been determined, that in addition to interacting with viral RNA; TMV-MP binds to components of the cytoskeleton (microfilaments) and to the ER in infected cells (Boevink and Oparka, 2005; Oparka, 2004; Reichel *et al*., 1999; Voinnet, 2005; Waigmann *et al*., 2004). Furthermore, viral particles can increase tenfold the exclusion limit of the plasmodesmata, facilitating viral movement (Hammond-Kosack and Jones, 2000). TMV-MP disappears distal from the infected cells, being undetectable six-cells away from the infection site. This indicates that the MP in the plasmodesmata at the point of infection is inactive (Oparka *et al*., 1997). It has been demonstrated that MP phosphorylation affects its activity (Trutnveva *et al.,* 2005; Waigman *et al.*, 2000). The MP phosphorylation seems to be mediated by a putative plasmodesmatal kinase linked to the cell wall (Citovsky *et al.,* 1993).

Recently, it has been suggested that microtubules are involved in MP degradation (Gillespie *et al*., 2002). Proteins linked to microtubules, like MPB2C and calreticulin could interact with TMV-MP (Chen *et al.,* 2005; Kragler *et al.,* 2003). Calreticulin is a chaperone protein located in the lumen of the ER that helps in protein degradation via the proteasome. It also participates in cell adhesion in animals (Coppolino *et al*., 1997). Over expression of this protein, increases the amount of MP associated to the microtubule. Hence, it has been speculated that it helps to remove the excess MP from the ER through the microtubules (Boevink and Oparka, 2005). TMV-MP, fused to green fluorescent protein (GFP), binds with the ER in early stages of infection (Gillespie *et al*., 2002; Heinlein *et al.,* 1998). On the other hand, TMV replicase 126K/183K associated to microfilaments is also necessary for viral cell-to-cell movement, and it has been observed linked to movement complexes (MCs: viral RNA complexes, MPs, and other viral and host proteins) (Hirashima and Watanabe, 2001 and 2003; Kawakami *et al*., 2004).

The virus particle reaches the vascular system from the companion cells, where it has direct access to the phloem (Carrington *et al*., 1996). Analysis of mutants of TMV and *Tobacco etch virus* (TEV) suggest that the capsid protein (CP) is necessary to allow virions to pass through the sieve elements to develop a systemic infection (Lazarowitz, 2000; Lazarowitz and Beachy, 1999). Some DNA viruses, apart from Geminivirus, also require the capsid protein for long distance movement (Boulton *et al*., 1989; Gardiner *et al*., 1988). Other viruses, i.e. Luteovirus, remain limited to the phloem, parenchyma, companion cells and sieve elements (Taliansky and Barker, 1999).

Nutrients and photo-assimilates are transported through the phloem. Therefore, viral infections reduce the absorption of these compounds in the apical leaves. The TMV-CP accumulates in the chloroplasts and associates with the thylakoid membrane producing misfolding in the ultra

structure of chloroplasts (Dawson *et al*., 1998). Moreover, it has been described that TMV associates with proteins of photosystem II, resulting in pigment degradation leading to the appearance of chlorotic symptoms in infected leaves (Lehto *et al*., 2003). Consequently, diseased plants often develop chlorosis and leaf distortion in the apical foliage regions. Some viruses can also systemically infect flowers and fruits, causing severe physiological damage to the host plants and high economical lost in fruit exporting countries (Herrera and Madariaga, 2002).

Host-virus interaction

Plants have developed recognition mechanisms that allow them to defend themselves against parasites (parasitic plants, insects, and some invertebrate animals) and pathogenic agents like viruses, viroids, bacteria, phytoplasms, fungi, and nematodes. Some of these mechanisms act as physical and chemical barriers that prevent infection by pathogens.

Compatibility and incompatibility reaction

Plants have developed a defence mechanism at the molecular level based on the gene for gene theory described by Flor (1971). This model is defined by the expression of a resistance gene (*R*) in the plant, which can bind directly or indirectly to the product of the avirulence gene (*avr*) of the pathogen (Bent, 1996; Ellis *et al*., 2000b). In this context, R proteins act as receptors and AVR ligands as elicitor proteins (Ellis *et al*., 2000b; Gabriel and Rolfe, 1990, 1990; Keen, 1990).

Table 1. Cloned and characterized virus plant resistance genes[1]

Gene	Host species	Virus[2]	AVR	Resistance mechanisms	Cloning method	Receptor structure	Reference
N	*N. tabacum*	TMV	Helicase domain of replicase	HR	Transposon tagging	TIR-NBS-LRR	Whitham *et al*., 1994
Rx1	*S. tuberosum*	PVX	CP	Replication	Positional cloning	CC-NBS-LRR	Bendahmane *et al*, 1999
Rx2	*S. tuberosum*	PVX	CP	Replication	Positional cloning	CC-NBS-LRR	Bendahmane *et al*, 2000
Sw5	*S. esculentum*	TSWV	MP	HR	Positional cloning	CC-NBS-LRR	Brommonschenkel *et al*., 2000
HRT	*A. thaliana*	TCV	CP	HR	Positional cloning	LZ-NBS-LRR	Cooley *et al*., 2000
RTM1	*A. thaliana*	TEV	nd	Systemic movement	Positional cloning	Jacalin like seq	Chisholm *et al*., 2000
RTM2	*A. thaliana*	TEV	nd	Systemic movement	Positional cloning	Jacalin like seq	Whitham *et al*., 2000
RCY1	*A. thaliana*	CMV	CP	HR	Positional cloning	CC-NBS-LRR	Takahashi *et al*., 2001
Tm22	*S. lycopersicum*	ToMV	MP	HR	Transposon tagging	CC-NBS-LRR	Lanfermeijer *et al*., 2003
Pvr21 *pvr22*	*C. annuum*	PVY	VPg	Replication, cell-cell movement	Approximation by homology	eIF4E	Ruffel *et al*., 2002
Mo11 *mo12*	*L. sativa*	LMV	nd	Replication. tolerance	Approximation by homology	eIF4E	Nicaise *et al*., 2003
Sbm1	*P. sativum*	PSbMV	nd	Replication	Approximation by homology	eIF4E	Gao *et al*., 2004

[1]Adapted from Kang *et al.,* 2005.
[2]CMV, *Cucumber mosaic virus;* LMV, *Lettuce mosaic virus;* PSbMV, *Pea seed borne mosaic virus*; PVY, *Potato virus* Y; PVX, *Potato virus* X; TCV, *Turnip crinkle virus;* ToMV, *Tomato mosaic virus*; TEV, *Tobacco etch virus*; TMV, *Tobacco mosaic virus*; TSWV, *Tomato spotted wilt virus*. nd, not determined.

In an incompatible reaction, development of the receptor-ligand complex triggers a cascade of transduction signals that ultimately leads to the HR response. The HR response is a local reaction characterized by programmed cell death at the infection site (Heath, 2000; Shirasu and Schulze-Lefert, 2003; Staskawicz *et al*., 1995). Furthermore during the HR, chemical oxidant species are produced (Lamb and Dixon, 1997), callose (Shimomura and Dijkstra, 1975) and lignin are synthesized, the levels of salicylic acid increase (Malamy *et al*., 1990; Naylor *et al*., 1998) and pathogenesis related proteins are produced (Yalpani *et al*., 1991). As a result, plants limit the short and long-distance movement of the pathogen.

Virus resistance genes

Currently, several viral resistance genes have been isolated, sequenced and characterized in diverse plant species (Table 1). For example, in tomato, the *Sw5* gene was identified by positional cloning and confers resistance to *Tomato spotted wilt virus* (TSWV). The SW5 receptor has a CC-NBS-LRR (*CC: Coiled Coil, NBS: Nucleotide Binding Site, LRR: Leucine Repeated Region*) structure. In Arabidopsis, *RTM1/ RTM2* genes give resistance to TMV and the *HRT* gene to *Turnip crinkle virus* (TCV). These genes also code for structural CC-NBS-LRR proteins (Cooley *et al*., 2000).

HRT gene is a single dominant gene, located on chromosome 5 that encodes a protein homologous to the RPP8 receptor which confers resistance to *Peronospora parasitica*. For this reason, they have been grouped in the *HRT/RPP8* family, although they recognize different pathogens (Cooley *et al*., 2000). Using transgenic *Arabidopsis* that express *HRT,* it was determined that this gene is insufficient to induce resistance to TCV. In the presence of *HRT* gene, transgenic plants activate HR response, but without the mediation of resistance. Full resistance to TCV is obtained when the recessive allele *rrt* is also present (Cooley *et al*., 2000). The TCV capsid protein is the elicitor of the HR response in this *HRT/RRT* system, interacting with the TIP (TCV *interacting protein*) transcription factor. It has been suggested that this interaction would serve to keep TIP out of the nucleus and avoid a molecular defence response by the plant (Ren *et al*., 2000).

In tomato, the product of gene *Tm22* recognizes *Tomato mosaic virus* (ToMV). It was isolated by transposon-mutagenesis and encodes the structural protein CC-NBS-LRR composed of 861 amino acids (Hall, 1980). The elicitor of *Tm22* is the movement protein (MP) (Weber *et al.,* 1993)

In *Arabidopsis,* ecotype C24 resists strain Y of *Cucumber mosaic virus* (CMV) (CMV-Y) due to the presence of the dominant *RCY1* gene (*Resistance to Cucumber mosaic virus Y strain*). *RCY1* gene is located on chromosome 5 of *Arabidopsis*, where other resistance genes are also found (i.e. *RAC3*, *RPS4*, *HRT*, *TTR*), including five different loci for *RPP* (Takahashi *et al*., 2001). Sequence analysis of this region, permitted the identification of the *RCY-1* gene, which encodes a 140 kDa protein with a CC-NBS-LRR structure. By creating chimeric viruses between CMV-Y and the virulent strain CMV-B2, the CP was identified as the avirulence factor of CMV-Y (Takahashi *et al*., 2001). *RCY1*-mediated resistance response requires transduction signals in which salicylic acid (SA) and ethylene participate (Takahashi *et al*., 2004).

Most virus resistance genes have an NBS/LRR structure in the carboxyl end. Small variations in the LRR domain allow changes in pathogen specificity (Ellis *et al.,* 2000a; Warren *et al.,* 1998). Usually *R* genes are monogenically dominant and trigger an HR response to viral infection. Nevertheless, the levels of expression of the receptor sometimes only allow an incomplete dominance (Kang *et al*., 2005). There are also examples of dominant or recessive genes that can induce a defence response against several species of a viral family. This has been reported for resistance gene *I* of *Phaseolus vulgaris,* which produces HR and defence to ten different viral species of the *Potyviridae* family (Fisher and Kyle, 1994).

In contrast, in species of *Capsicum* a defence response to *Pepper veinal mottle virus* (*Potyvirus*) occurs only if the plant alleles *pvr12* (*elF4E* homologue) and *pvr6* (*elF(iso)4E*) are homozygotic (Caranta *et al*., 1996). A similar situation has been reported for the *Rx* gene and *rrt* allele (Bendamahne *et al*., 1999; Cooley *et al*., 2000) in potato, conferring resistance to PVX.

Virus variability

Plant viruses can mutate and evolve quickly. This may be favored by the presence of several viral genomes in each infected plant cell and by the short replication cycles. The replicase of RNA virus lacks repairing activity, increasing mutations rate to 10^{-4} per replication cycle for each base.

In addition to mutations, viruses have a genetic variation due to recombination and the acquisition of additional genomes. These characteristics grant them the ability to modify *avr* genes and eventually to overcome the defence barriers of host plants.

Post-transcriptional gene silencing

Viruses have been able to overcome very complex defensive barriers developed by hosts. In the 90's, an extreme type of defence consisting of silencing viral RNA was described. Today, it is known as RNA interference (RNAi) and post-transcriptional gene silencing (PTGS) in animals and plants, respectively. Generally, plants have this silencing mechanism for several factors; for example, to control virus infection (Baulcombe, 2000; Carrington, 2000).

Virus gene silencing begins with the identification of the RNA duplex formed between the positive (sense) and negative (antisense) strand viral RNA (Hamilton and Baulcombe, 1999). This structure is generated as an intermediary during the replication of the positive-stranded viral RNA. A multi-component complex, including RNA dependent RNA polymerase (RdRp), RNA helicase and DICER/RISC, degrades the RNA in small fragments of 21-27 nucleotides (RNAi). These molecules are responsible for amplifying and transmitting the silencing signal to the rest of the plant (Jana *et al*., 2004; Mlotshwa *et al.,* 2002; Hammond *et al.,* 2001; Hamilton and Baulcombe, 1999).

This endogenous strategy has been subsequently used to control virus infections in plants, and it has been applied to restrict TEV infections (Lindbo, 1993). Efficient induction of PTGS can be achieved by incorporating antisense viral DNA into the plant as a transgene (Ding *et al*., 2004). The host plant in contact with the corresponding virus translates and replicates the viral proteins. However, soon the transcript level decreases due to the formation of the RNA duplex between the positive virus strand and the negative transgene strand. Viral accumulation gradually decreases without causing considerable damage to the host plant.

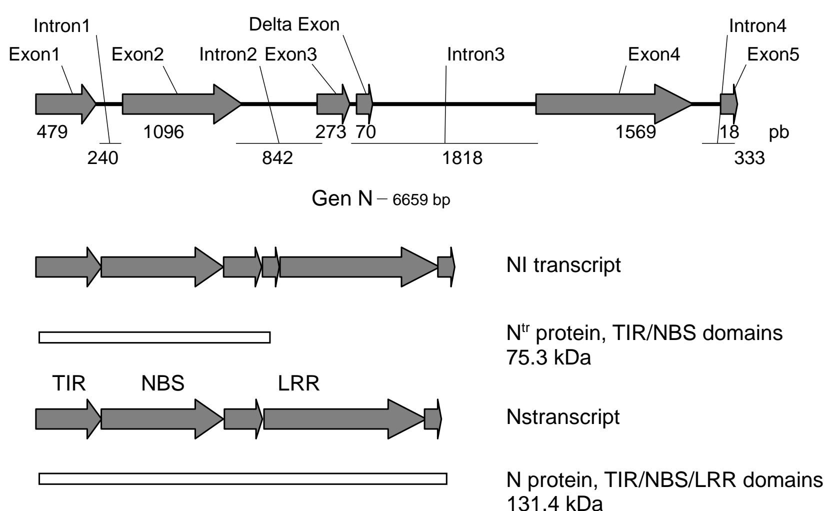

Figure 1. Schematic representation of the genomic sequence of the N gene. The genomic sequence of N gene is 6659 pb long and contains 5 exons and 4 introns. The Ns transcript and the Nl transcript produced trough alternative splicing are shown. According to the deduced amino acid sequence of the N receptor, a TIR and NBS domains are found at the N-terminus of the protein. The C- terminal end contains a leucine rich repeat (LRR) domain.

Viruses have been able to co-evolve and some *Potyvirus* and *Tobamovirus* have plant silencing suppressor proteins (Li and Ding, 2001). For instance, the suppressor protein P1/Hc-Pro (*Helper component-proteinase*) is coded in the genome of *Potyvirus* and the suppressor protein 2b is coded in the genome of *Cucumovirus* (Kasschau and Carrington, 1998; Kasschau *et al.,* 1997; Li *et al.*, 1999).

The Hc-Pro protein is a potent inhibitor of the gene silencing mechanism at the infection site. However, it incompletely eliminates the escape of the mobile signal (RNA of 25nt) to healthy tissue (Mallory *et al.*, 2001). For this reason, the viral infection still occurs but at a reduced rate. This characteristic makes TEV, PVX and PVY virus very aggressive when they invade the host and it might be an inheritable characteristic of other viral genomes.

N gene confers resistance to TMV in tobacco.
The interaction between TMV-U1 and the product of *N* gene present in *N. tabacum* has been a classical model for the study of viral defence responses. The *N* gene was described in *N. glutinosa* and it was later transferred to *N. tabacum* using classical plant breeding techniques to confer resistance to *Tobamovirus* genus in commercial cultivars of tobacco.

The *N* gene was isolated by mutagenesis of TMV-U1 resistant *NN* tobacco plants. This was accomplished using the corn transposon activator (Ac) in *N* gene carrier plants (Whitham *et al.*, 1994). Genomic DNA sequence analysis demonstrated that there are 5 exons and 4 introns (Figure 1). Furthermore, it was proved that the immature transcript undergoes alternative splicing at intron 3, as in other *R* genes of the TIR/NBS/LRR family (Jordan *et al.*, 2002). Two mRNAs are generated from the alternative splicing. Firstly, a longer transcript (Nl) encodes a truncated protein (N^{tr}) of 652 amino acids (75.3 kDa). Production of this smaller protein is due to the presence of a stop codon located in the exon (from intron 3) generated by alternative splicing (Figure 1). It was determined using RT-PCR that the virus induces alternative splicing in the N gene (Dinesh-Kumar *et al.*, 2000).

The second transcript (Ns) is preponderant and encodes the complete N protein of 1144 amino acids (131.4 kDa). This protein possesses in its amino terminal region (8-150 amino acids), a TIR or CD (cytoplasmatic domain) domain with 49 and 55% homology with the interleukin 1 (IL1R) receptor and with the amino terminal of the receptor Toll from *Drosophila*, respectively. This domain is found in exon 1 (479 bp) of the *N* gene. Exon 2 encodes an NBS motif, which contains the P-loop, kinase 2 and kinase 3a motifs. These could be required to bind ATP or GTP nucleotides, necessary for protein phosphorylation. The LRR domain begins in exon 3, although it is mainly coded by exon 4. This domain has 14 repeats of consensus LxxLxLxxN/CxL, of 26 amino acids with intervals of leucine. Exon 5 encodes the last five amino acids of the N protein.

The fact that native N protein lacks a signal peptide or transmembrane domains, suggests that N is a cytoplasmatic receptor (Whitham *et al.*, 1994), which is consistent with the location of TMV replication in cell cytoplasm (Dawson, 1992).

It has been determined that the expression of the full N gene in tomato or tobacco normally lacking this gene, is necessary and sufficient to confer resistance to TMV (Whitham *et al.*, 1996). Protein N^{tr} is necessary to trigger a complete HR reaction in tobacco that carries the *N* gene. Without this truncated N^{tr} protein, the plants develop an incomplete resistance response to TMV-U1 (Dinesh-Kumar and Baker, 2000). The resistance response fails, generating local necrotic lesions and the plant is also incapable of avoiding the systemic movement of the virus (Dinesh-Kumar and Baker, 2000).

Induction of HR in *NN* tobacco plants takes place in response to infection with TMV-U1 and all other viruses of the *Tobamovirus* genus. In general, HR manifests below 28 ºC. Above this temperature, the HR response is inhibited and the virus disseminates systemically in the plant (Weststeijn, 1981). When the temperature falls below 25 ºC, HR is reestablished, causing a generalized cellular death, due to the systemic recognition of the virus in the plant.

Thermosensitivity was analyzed with hybrid

viruses, leading to the proposal that temperatures above 28 ºC weaken the interaction between the virus elicitor and receptor. This avoids the induction of the defence response and the development of HR in the host plant (Padgett *et al.*, 1997).

Receptor N and its interaction with TMV-U1 replicase, a possible mechanism of action
TMV-U1 is one of the 16 viruses of the *Tobamovirus* genus that infects mainly Solanaceous plants. *Tobamovirus* are positive single-stranded RNA virus wrapped by capsid proteins (CP). Morphologically, they are rigid rods 300 nm long. These viruses can passively penetrate the plant through cell injuries.

In the host cell, the virus particle dissociates and the proteins encoded in four open reading frames (Dawson 1992) are translated. A 126 kDa protein is encoded at the 5' end and a 183 kDa protein may be obtained due to the presence of an amber codon. Proteins 126 kDa and 183 kDa act as replicases and contain the methyltransferase and helicase domains. Replicase 126kDa/183kDa are needed to replicate the viral RNA that is utilized for translation with the cell translation machinery. The capsid protein (CP) of 17kDa, the movement protein (MP) of 30 kDa and a protein of 54 kDa are synthesized from the subgenomic mRNAs inside the infected cell (van Regenmortel and Meshi, 1995).

It was demonstrated using TMV-U1 chimeric viruses, that the helicase region of replicase is the AVR factor needed for HR induction (Padgett *et al.*, 1997). It has been determined that the elicitor of TMV-U1 is a 50 kDa region of helicase domains (Abbink *et al.,* 1998; Erickson *et al.*, 1999). Similar to the TMV-U1, the result obtained with chimeric viruses was thermosensitive and N gene dependent. Also, it was demonstrated that viral helicase has ATPase activity, but this activity is not required to induce HR (Erickson *et al*., 1999).

The precise interaction between the elicitor and the N receptor, during infection with TMV-U1, is still unknown. Therefore, it has been postulated that additional host factors may also be involved in this defence mechanism. The NRG1 protein was recently identified using PTGS. This protein has a CC-NBS-LRR structure, and together with receptor N, seems to be involved in resistance to TMV (Peart *et al.*, 2005). Furthermore, some host proteins form complexes before infection in the absence of the pathogen. R proteins probably act as guardians, recognizing the AVR product through this preformed complex. Several studies with *Pseudomonas syringae* resistance receptor RPM1 support this hypothesis (Leister and Katagiri, 2000; Mackey *et al.,* 2002; Mackey *et al.,* 2003).

N. benthamina co-expressing Rx receptor domains has enabled researchers to study the interaction mechanisms between the plant receptor and the pathogen elicitor. This demonstrated the generation of intramolecular interactions between the LRR and CC domains in Rx, which break in the presence of the PVX elicitor (Moffet *et al.,* 2002). Rx mutants, with a modified P loop motif (G175A, K176A) in the NBS domain, inhibit the capacity of Rx to induce HR (Bendamahne *et al.,* 2002). However, this mutation does not alter the *in vitro* interaction between the LRR and the CC-NBS domains of the receptor (Moffet *et al*., 2002). This indicates that the interactions between CC and LRR domains with the NBS domain are different from each other. Furthermore, the activation of Rx depends on the separation of LRR and NBS domains (Moffet *et al.,* 2002). This evidence indicates that before virus infection, the LRR domain acts as a negative regulator of the defence response mediated by the receptor.

Extending these results to the mechanism of defence induced by the N receptor, it has been proposed that the recognition of the helicase (p50) region of the 126 kDa/183 kDa replicase of TMV-U1 takes place via the LRR domain of the N receptor and that host proteins, such as NRG1 and N^{tr} are needed to recognize the elicitor and activate the defence response. In this regard, it is important to recall that the N receptor requires both the complete N and the truncated N^{tr} proteins to trigger the HR (Dinesh-Kumar and Baker, 2000).

When the host plant is infected with TMV-U1, it induces alternative splicing in intron 3 of the *N* gene. This enables the synthesis of protein N^{tr} in balanced proportions during the infection

process (Dinesh-Kumar and Baker, 2000). From this result, one may infer that variant N^{tr} (protein TIR/NBS) interacts with receptor N and possibly with other host proteins, e.g. NRG1 (CC/NBS/LRR), to mediate a coordinated defence response.

Recently, the *NH* gene was cloned and characterized. This gene is a gene homolog to the *N* gene that is present in TMV resistant and sensitive tobacco plants (Stange *et al.*, 2004). Sensitive tobacco plants that have the *NH* gene produce an HR-like response to TMV-Cg, a TMV strain that preferentially infects crucifer plants (Eherenfeld *et al.*, 2005; Stange *et al.*, 2004; Yamanaka *et al.,* 1988). In spite of this local defence response, the virus moves systemically. It was determined that the *NH* gene does not have the alternative splicing site in intron 3; hence the NH^{tr} protein is not produced. It is suggested that the absence of NH^{tr} could be the cause of the unsuccessful defence response to TMV-Cg (Stange *et al.*, 2004).

In animal cells, the LRR domain mediates protein-protein interactions between the pathogen RpRd and the PRI (protein ribonuclease inhibitor) encoded by the host. The LRR domain adopts a horseshoe like tertiary structure in PRI, due to the presence of 29 LRRs (Kobe and Deisenhofer, 1995). The LRRs present in the plant pathogen receptors have 20 to 26 LRRs allowing this domain to acquire a beta folded structure of half a horseshoe (Yoder *et al.*, 1993). In the cytoplasmic receptors, like N, the LRR domain would confer ligand recognition specificity (Jones and Jones, 1997; Kobe and Kajava, 2001), perhaps by permitting the dimerization with the N^{tr} protein or to other components that participate in the transduction pathway induced by the viral signal recognition (Hammond-Kosak and Jones, 1997).

The presence and requirement of TIR/NBS truncated proteins in defence response in plants is proposed by a model in Figure 2. At the same time that R recognizes the elicitor, it interacts with host proteins, to carry out a primary response of intracellular signaling. Regarding the N receptor, this activation would be

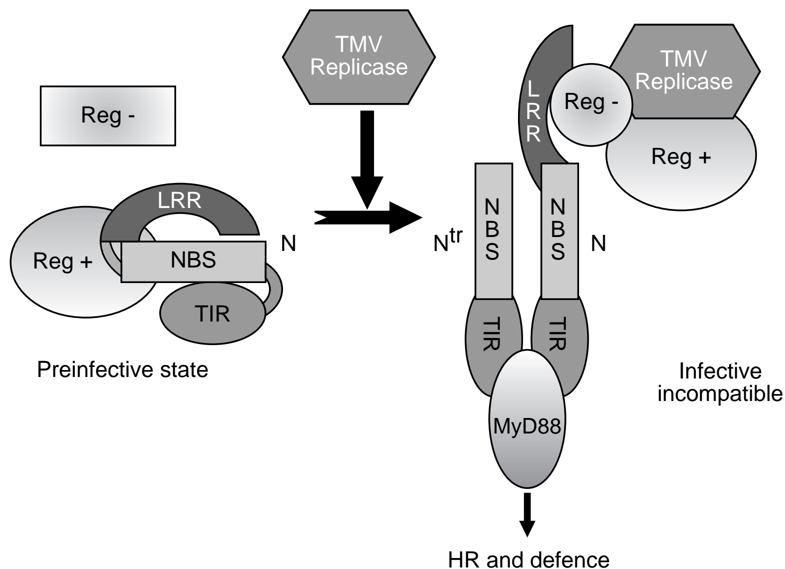

Figure 2. Hypothetical model of a preinfective and infective incompatible state in a N-TMV interaction. The N receptor would be inactive in a preinfective state and associated with positive (Reg+) and negative (Reg-) HR factors. Once the infection develops, the AVR elicitor (replicase) is synthesized within the cell, binds to host negative or positive HR factors and indirectly with the N receptor. The viral AVR recognition evolves an initial defence response that induces the alternative splicing of N gene that results in the expression of two proteins, N and Ntr. The Ntr protein does not contain an LRR domain, but could heterodimerize with N receptor or/and other adapter proteins like MyD88 or NRG1, through the TIR domain to induce a complete defence response (Dinesh-Kumar and baker, 2000; Stange, 2004)

necessary and sufficient to induce the expression of the receptor itself and of the adapting truncated protein. It is possible that once the N^{tr} protein is recruited by receptor N, signals that are more persistent are triggered to induce the complete defence response. Signaling of molecular events induced from the generation of receptor-N^{tr}-elicitor complex should decrease later. This may be due to the transient synthesis of protein N^{tr}. The molecular mechanism, by which this process is carried out, is still unknown.

This information supports the importance of LRR domain in establishing the HR response. However, this does not provide evidence that this domain interacts directly with the virus elicitor. Independent of the type of interaction between the N protein and the elicitor, signal transduction episodes could be triggered. This would include protein phosphorylation through the TIR domain, similar to the process that has been described in animal cells for TLR (Toll Like Receptors), RIL1 in mammals and in Toll of *Drosophila* cells (Muzio *et al.,* 2000; Quershi *et al.,* 1999; Schneider *et al.,* 1991).

In mammalian cells, out of 10 TLRs described, TLR2 and TLR 4 are the most extensively characterized. These receptors recognize the LPS (Lypopolysacharide) and peptidoglycan of gram-negative bacteria. These receptors have a variable region in the TIR domain that enables homodimerization, and a conserved region involved in the heterodimerization to other proteins with TIR domains (i.e. MyD88). Interaction with this adapting molecule enables signal triggering through serine/threonine protein kinase IRAK that finally converges in the translocation of transcription factor NF-kB from the cytoplasm to the nucleus and the binding to IkB regions located in promoters of immune activated genes. This event enables the activation of gene transcription involved in the immune and inflammatory response (Bauerle, 1991).

As in the mechanism described previously, it has been proposed that recognition of TMV-U1 helicase (virus elicitor) by the N receptor could activate transcriptional factors. This ultimately would be reflected in HR production (Dinesh-Kumar et *al.,* 1995).

Currently, IkB analog proteins have been found in *Arabidopsis* and tobacco. The NPR1/NIM1 protein that is activated by salicylic acid revealed the presence of 4 ankyrin repeats, homologous to those found in IkB in mammals and in the cactus protein of *Drosophila* (Cao *et al*., 1997; Ryals *et al*., 1997). Ankyrin IkB motifs are essential for their interaction with NF-kB, so it has been proposed that NPR1/NIM1 interacts with other proteins, possibly transcription factors, through these repeats.

Sustaining this hypothesis, TGA family type bZip transcription factors have been shown to interact with NPR1 (Kim and Delaney, 1999; Zhou *et al.,* 2000). TGA factors would recognize the TGACG motif present in the defence promoter gene of pathogenesis related proteins (PR) and glutathione-S-transferase (GST) (Kim and Delaney, 1999). NPR1 mutants, in whom the ankyrin domain has been eliminated, fail to bind TGA factors, resulting in the loss of ability to induce PR expression (Zhou *et al*., 2000). Mou *et al*., 2003 determined that NPR1 remains in the cytoplasm forming oligomers generated through disulphur bond interactions. In response to the accumulation of salicylic acid, the defence response is activated, and antioxidant compounds are synthesized. Under these reducing conditions, NPR1 dissociates to a monomeric state, by reducing the disulphur bridges. This induces the translocation of NPR1 monomers to the nucleus to activate PR gene expression during HR. Using virus induced post-transcriptional gene silencing (VIGS), it was established that NPR1 is an essential factor for the defence route mediated by the N receptor in TMV resistant tobacco (Liu *et al.,* 2002b).

By means of VIGS it was also determined that the EDS1 protein is necessary for receptor TIR/NBS/LRR mediated resistance, among which is receptor N (Falk e*t al.*, 1999). EDS, cloned and characterized in 1999, encodes a protein highly homologous to eukaryotic lipases. This enzyme could mediate the hydrolysis of lipidic molecules during HR. EDS1 seems to act downstream of salicylic acid and would be necessary for the accumulation of PR1

messenger (Falk *et al.*, 1999). Furthermore, components of the protein degradation pathway participate prior to the oxidative burst and cell death generated by the interaction of the N receptor with TMV-U1. The involvement of Rar-1 was verified using the yeast two hybrid system. Rar-1 is a protein with a zinc-finger motif that interacts with factors of the multiproteic COP9 and SCF complex for proteic degradation through ubiquitination (Liu *et al.,* 2002a). Likewise, the importance of Rar-1 and of COP9 and SCF in the complex in the defence response mediated by the N receptor was demonstrated through the VIGS system. Based on recent information, it was possible to associate the N receptor to the Rar-1-COP9-SCF complex, which would bind proteins with F–box domains and thus target such factors for degradation. Thus, negative regulators of the defence response would be degraded through the COP9-proteasome complex (Liu *et al.,* 2002b).

Recent studies performed with *Arabidopsis thaliana*, demonstrated that MAPK activation takes place before the increase of reactive oxygen species (ROS), in the HR induction route (Ren *et al.*, 2002). A few years ago, the participation of MAPK, SIPK (*salicylic acid-induced protein kinase*) and WIPK (*wounding-induced protein kinase*) was described in the N mediated defence route. Transcript levels of these kinases increased in Xanthi NN tobacco plants infected with TMV-U1 (Zhang and Klessig, 1998). *In vitro* trials have revealed the participation of NtMEK *(Nicotiana tabacum map kinase kinase)* that would be responsible for phosphorylation and interaction through its amino terminal domain with SIPK and WIPK (Jin *et al*., 2003).

Concluding remarks

In almost half a century, it has been possible to partially understand the complex interaction that is established at a cellular level between the host plant and the virus.

Several host plant proteins participate during the viral cycle. Some of these proteins (i.e. microtubules, filaments of actin/myosin, calreticulin) facilitate the infective process and virus movement through the plant. Others, like the receptors encoded by resistance genes, interact with viral proteins in the virus recognition process. The recognition of the pathogen by the host plant induces a hypersensitivity reaction (HR) and a systemic defence. This is unfavorable for the development of the virus cycle, avoiding massive and systemic virus dissemination in the host plant.

Viruses can develop new races, with variants in AVR proteins and silencing suppressor factors. This allows the virus to circumvent molecular recognition barriers, eventually developed by the host plants.

In this direction, it has been possible to identify the *eIF4E* gene (translation initiation factor 4) as a recessive resistance gene to *Potyvirus* after discovering that *eIF(iso)4E* protein interacts with VPg protein from ToMV. This finding was subsequently used to protect cereals from virus infections (Gao *et al*., 2004; Nicaise *et al.*, 2003; Ruffel *et al*., 2002).

The inclusion of a viral gene fragment in a susceptible plant is another feasible technique to use in the development of new virus resistant crop plants. In this case, after the host plant has been infected, cascades of reactions, associated with the development of post-transcriptional gene silencing (PTGS) are induced, avoiding virus multiplication and reducing host plant damage.

The over-expression of genes associated to more than one *R* gene has also been used in the study of virus resistance. For example, over-expression of the *NPR1* gene under the 35S CaMV (Cauliflower mosaic virus) constitutive promoter increases resistance to several bacterial pathogens. It is interesting to note that resistance to pathogens may be obtained by increasing the expression of an intermediary protein (e.g.: NPR1). In this aspect, additional research is needed to define the factors involved in transduction of signals generated during plant-virus interactions. This knowledge will allow the determination of additional host factors that participate in the resistance mechanisms induced

by one or more viruses.

Other lines of research are the identification of *R* genes in plant models and in agronomically important species. Thus, in a wide range of species, resistance gene analogues (RGA) have been obtained through PCR amplification of conserved regions of resistance genes, such as NBS or LRR domains. This approach has been successfully applied to isolate NBS-LRR genes from several monocot and dicot species (Shen *et al*., 1998). Using degenerate primers of the NBS domain, RGAs from a broad range of plant species like citrus (Deng *et al*., 2000), grapevine (Di Gaspero and Cipriani, 2002) and apple (Balde *et al*., 2004) have been amplified. In apricots, RGA sequences associated to *Plum pox virus* (PPV) resistance were cloned and characterized (Dondini *et al*., 2004, Soriano *et al*., 2005).

Development of genetic maps with RGA markers may be the appropriate strategy to identify genomic regions associated to resistance genes (Quint *et al*., 2002; Soriano *et al*., 2005).

Nevertheless, the identities of all host factors involved in the viral cycle are still unknown. This is one of the greatest challenges in plant virology today. A greater knowledge is needed to facilitate the development of genetic programs orientated to obtain virus resistance in different crops. At the same time, it will be necessary to overcome several governmental barriers related to the use and consumption of genetically modified agricultural products.

Resumen

Los virus que infectan plantas son generalmente de tipo DNA o RNA de cadena simple y positiva. El ciclo viral se inicia al penetrar el virus en la célula hospedera. Este comienza con el desensamblaje, replicación del RNA, traducción de proteínas, ensamble, liberación, movimiento de célula a célula y a larga distancia. El conocimiento de los mecanismos de interacción entre la planta hospedera y el virus, ha progresado considerablemente en los últimos treinta años. Por ejemplo, se ha determinado la participación de componentes del citoesqueleto y de proteínas del hospedero en movimiento local (célula a célula) y a larga distancia (movimiento sistémico) de los virus en las plantas. Además, se han caracterizado numerosos receptores virales codificados por genes de resistencia (*R*) y se ha determinado el mecanismo de defensa en *Arabidopsis thaliana* y en especies de las familias Solanaceae, Cucurbitaceae y Leguminoseae. Esto ha contribuido considerablemente a comprender la compleja interacción planta-virus. La mayoría de los genes *R* descritos poseen dominios de consenso como LRR, NBS, TIR y LZ. Esto sugiere convergencia en los mecanismos de transducción de la señal de defensa. Los virus evolucionan rápidamente debido a cortos ciclos de replicación y a la existencia de muchos genomas en cada célula; esto a través de numerosas células en cada hospedero y numerosas plantas hospederas infectadas. Por esto, los virus han generado variantes de genes de avirulencia, lo que les permite sortear las barreras moleculares de defensa en plantas. Como estrategia para superar la aparición de nuevas razas de virus, las plantas generan nuevos genes *R* mediante procesos de recombinación. También pueden desarrollar mecanismos de defensa alternativos especializados, como silenciamiento génico post-transcripcional. Sin embargo, algunos virus (ej. *Potato virus X*), son capaces de suprimir el silenciamiento viral post-transcripcional en el hospedero. En esta revisión se describen recientes descubrimientos de la interacción planta–virus y se presenta como modelo, la respuesta de defensa desencadenada en *Nicotiana tabacum* portadoras del gen *N*, el que otorga resistencia a *Tobacco mosaic virus*. Se proponen mecanismos de transducción, que activan la cascada de eventos moleculares, que conllevan finalmente a la respuesta de defensa a virus en las plantas.

Palabras clave: Gen *N,* genes de resistencia, mecanismo de defensa, movimiento viral, replicación viral, virus, TMV.

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