

Minireview Paper

Immune Response of *Lycopersicon esculentum* against the Tomato Mosaic Virus

ABSTRACT

The tomato plant (*Lycopersicon esculentum*) is a crop rich in nutriment but is susceptible to infection such as *mosaic* caused by the Tomato Mosaic Virus (ToMV). In this review it will be described some mechanisms that *L. esculentum* have developed to overcome the infection amongst ToMV such as the Hypersensitive Response in which participate the resistance proteins Tm-1, Tm-2, and Tm-2², as well as the Antiviral RNA Silencing.

Keywords: ToMV, Mosaic, R proteins, Antiviral RNA Silencing

1. INTRODUCTION

The tomato plant (*Lycopersicon esculentum*) is a crop rich in carotenoids, polyphenols, folate, vitamin E, vitamin C and other several water-soluble vitamins, trace elements, phytosterols, and potassium [1,2]. Because of these nutrimental properties, tomato has been included in the worldwide diet. Furthermore, it has been observed that its consumption have beneficial effects on blood pressure and apolipoprotein apoA-I levels being beneficial for reducing cardiovascular risk [3]. Thus the tomato crop is of great importance in agriculture, being the second most important with a worldwide production of 100 million tons of fresh fruit [4].

Tomato is cultivated in different geographical areas with changing environmental conditions. This situation implies that crops are susceptible to develop diseases caused by viruses, bacteria and fungi representing quality and monetary losses. In this review it will be analyzed the immune response that *L. esculentum* triggers in order to counteract the Tomato Mosaic Virus (ToMV), the etiological agent of one of the principal diseases that affects tomato crops, *The Mosaic*.

2. TOMATO MOSAIC VIRUS

The ToMV is a biotrophic pathogen transmitted mechanically mainly infecting tomato plants systemically causing mosaic symptoms characterized by the alternation of chlorotic and dark green areas as well as leaf mold (Figure 1) [5,6].



Figure 1. *Lycopersicon esculentum* infected with ToMV. Chlorotic and green areas are appreciated as well leaf mold.

The ToMV belongs to the genus Tobamovirus of the family Virgaviridae. It has a single-stranded RNA positive-sense genome [5] of around 6.3 Kbp. It includes four open reading frames (ORFs) coding for the 130- and 180-kDa replication proteins, the movement protein, and the coat protein [7,8].

The 130- and the read-through 180-KDa proteins have two domains, the methyltransferase (MT) and the helicase (Hel); the first is involved in the 5' capping of viral RNAs. The 180 KDa protein possesses in its C-terminal region the activity of an RNA-dependent RNA polymerase (RdRP). In addition, both proteins are implicated in the viral cell-to-cell movement [9,10].

The cell-to-cell movement protein (MP; ~30 KDa) is encoded downstream of the replication proteins and is expressed from the respective sub genomic mRNA during infection [9]. The MP interacts *in vitro* with single stranded nucleic acids giving rise to a ribonucleoprotein that has been proposed to facilitate the intercellular passage through plasmodesmata in order to disperse the infection [11].

The coat protein (CP; ~17 KDa) is encoded downstream of the MP and synthesized during infection from a sub genomic mRNA encompassing the 3' region of the viral genome [12]. Apart of provide protection to the genomic RNA, the CP seems to play a role in the establishment of the virus replication complex but seem to be dispensable for replication [13].

2.1 REPLICATION OF ToMV

It has been determined that either host intact membranes as well as host proteins are necessary to the formation of the virus replication complex [14]. In the case of Tobacco Mosaic Virus (TMV) -other

Tobamovirus- replication complexes are formed on the endoplasmic reticulum membrane [13]. Regarding host proteins, TOM1, TOM2A, ARL8, the eukaryotic Elongation Factor 1A (eEF1A), and the Heat shock protein 70 (Hsp70) are associated with the replication proteins associated to membranes [14,15].

It has been proposed that once inside the cytoplasm translation of ToMV RNA is achieved and the replication proteins 130- and 180-KDa are synthesized. During translation, these proteins bind to the genomic viral 5' UTR giving rise to a pre-membrane-targeting complex (PMTC). The PMTC is able to bind to the surface of membranes through the replication proteins which in turn join to the host proteins TOM1 and ARL8 in order to initiate the synthesis of the negative strand [9, 16]. It has been proposed that RNA synthesis begins until the template RNA is isolated from cytoplasm in order to avoid the recognitions of the double stranded RNA produced during replication by the host silencing machinery that would destroy it. In agreement with this, it seems that the RdRP activity of replication proteins is acquired until they are associated to the membrane [14].

3. IMMUNE RESPONSE IN *Lycopersicon esculentum*

Growing under different environmental conditions *L. esculentum* is exposed to infectious agents such as the ToMV but is able to trigger an immune response in order to counteract the infection. Different from animals, plants do not possess an immune system based on mobile cells, instead they trigger a molecular signal immunity activated by pattern-recognition receptors (PRRs), by resistance proteins (R), and the RNA silencing machinery. The plant-virus interaction elicits mainly the last two (Figure 2).

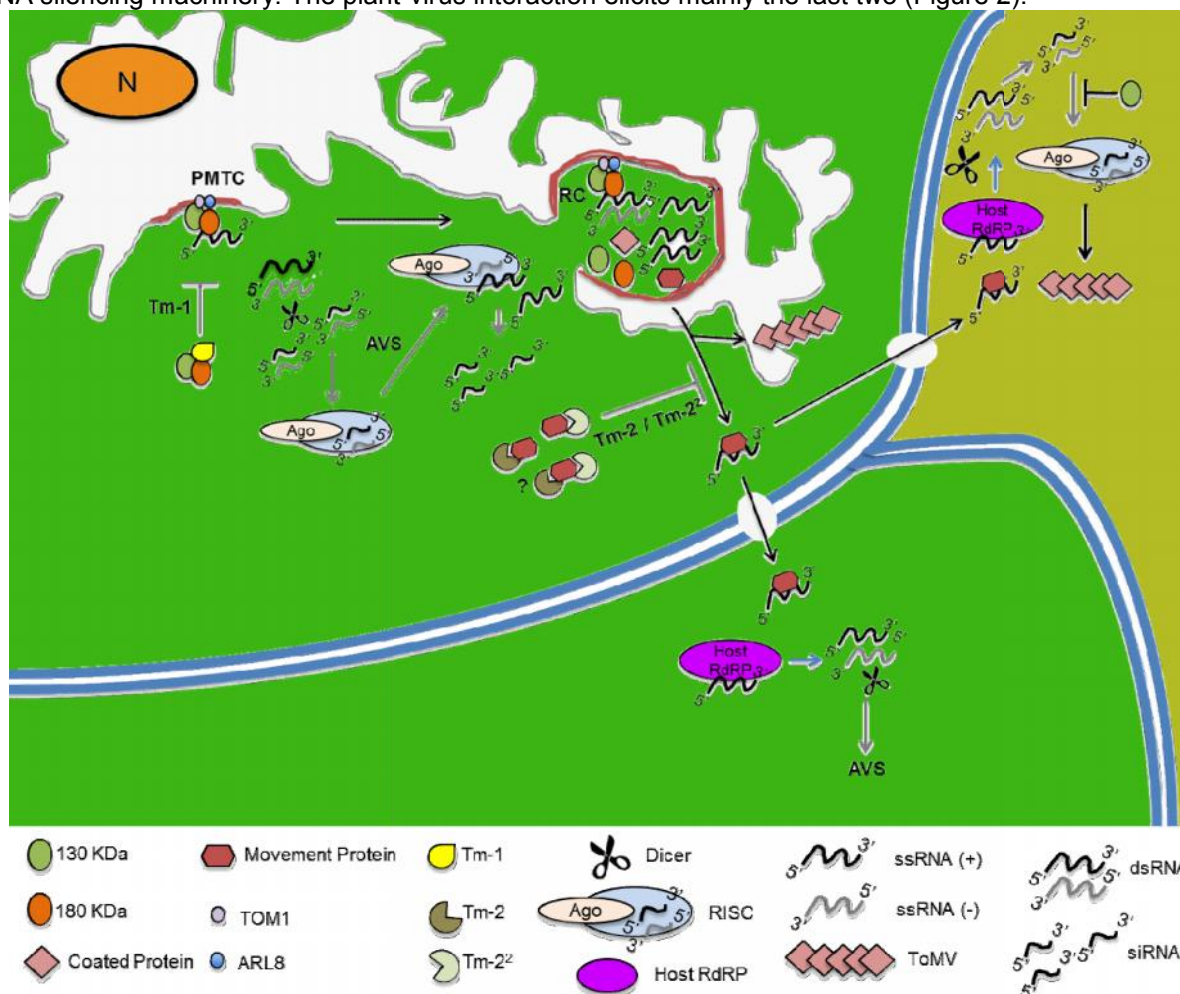


Figure 2. Immune Response of *L. esculentum* against the ToMV. ToMV replicates its genome in replication complexes (RC) whose precursor is a pre-membrane targeting complex (PMTC) in which the

replication proteins (130- and 180- KDa) are recruited and associated with host proteins (TOM1 and ARL8). This step is inhibited by Tm-1. If viral RdRP activity is initiated before formation of the RC, synthesis of viral dsRNAs are produced by Dicer and are prone to be processed by the antiviral RNA silencing (AVS) machinery. Spread of AVS is through plasmodesmata and its amplification is mediated by host RdRPs. In some cases AVS is overcome by suppressor viral molecules such as the 130 KDa. This event is associated with chlorotic areas (yellow-green color in the figure) while non-infected tissues are green. Resistance proteins Tm-2 and Tm-2² bind the effector movement protein, it is unknown how this interaction is (symbol "?" in the figure). These proteins conduct to a hypersensitivity response accompanied by programmed cell death. White zone in the figure represents the Endoplasmic Reticulum. N: nucleus.

3.1 IMMUNITY ACTIVATED BY PATTERNS RECOGNITION RECEPTORS

Pathogen or Microbial Associated Molecular Patterns (PAMPs or MAMPs) are conserved structural elements of pathogenic and non pathogenic microorganisms such as the peptidoglycan structure present in the bacterial cell wall, the lipopolysaccharides in Gram-negative bacteria, and oligosaccharides as well as chitin and its derivatives from the cell wall of fungi. When PAMPs/MAMPs are recognized by Pattern Recognition Receptors (PRRs) the first line of defense in plants called MAMP-triggered immunity (MTI) is activated. Until now viral MAMPs have not been described [6,17].

3.2 THE GUARD HYPOTHESIS

As a second line of defense, a plant elicits the Effector-Triggered Immunity (ETI) in which receptors known as resistance (R) proteins play a pivotal role [17]. According to the "guard" hypothesis, R proteins are activated when a self-protein is altered by an effector pathogen molecule (the "avirulence" signal) [18]. In response to the avirulence signal plants trigger a hypersensitive response (HR) frequently associated with programmed cell death at the site of invasion resulting in necrotic lesions, as well as the production of antimicrobial molecules. In some cases the HR is not activated [19].

R proteins include members of the NB-ARC-LRR family (Nucleotide Binding-ARC/Leucine-Rich Repeat) [6,17]. They consist of the effector, the STAND and the LRR domains. The effector domain in the N-terminal region is characterized by the presence of protein interacting domains such as the coiled coil (CC); the STAND domain (Signal Transduction ATPase with Numerous Domains) is characterized by the NB-ARC region (Nucleotide-Binding adaptor shared by APAF-1, certain R gene products and CED-4) which is thought to be involved in the switch that dictates the activation state of the R protein; finally the LRR (Leucine-Rich Repeat) domain is responsible for the pathogen sensing [20,21,22].

During ToMV infection *L. esculentum* switches on the ETI response through the CCNB-ARC-LRR proteins Tm-2 and Tm-2², but also through other class of resistance proteins such as Tm-1 [23,24]. Genes coding for these proteins have been introduced in cultivated species of tomato in order to control ToMV infection.

3.2.1 Tm-2 and Tm-2²

Tm-2 and Tm-2² are R proteins of 891 amino acids (~98 kDa) that belong to the CCNB-ARC-LRR protein family. They differ only in four amino acid residues; two in the NB domain and two in the LRR domain. These proteins are codified by allelic forms that differ only in 7 nucleotides [25,26]. Tm-2 recognizes a domain in the N-terminal region of the MP protein while Tm-2² requires recognition of a domain in the C-terminal region. Therefore, even though they activate the ETI response, it might be possible that they trigger different mechanisms of resistance. According to this, it has been observed that Tm2² interaction with the MP is more sensitive when amino acid changes are present in the latter compared with Tm-2-MP interaction [25,27].

3.2.2 Tm-1

The Tm-1 protein does not belong to the NB-ARC-LRR protein family, it has different characteristics, it

has a TIM barrel structure. *Tm-1* is a semidominant gene that is formed by nine exons and eighth introns and codes for a protein of 754 amino acids (~80 KDa). From this gene results another splice variant by skipping of the second exon but it does not inhibit the ToMV replication [23].

Tm-1 cellular role is unknown, but it inhibits ToMV replication. It has been observed that *Tm-1* is recruited to the membrane together with the replicative complex; also inhibits the association of the host proteins to the PMTC, when it occurs synthesis of the negative-RNA is not carried out [15,16,23].

3.3 ANTIVIRAL RNA SILENCING

The RNA silencing mechanism controls gene expression through the endonucleolytic cleavage of transcripts or their translation inhibition [28]. A double stranded RNA (dsRNA) structure starts the process when it is recognized and cleaved by an RNase III family endoribonuclease (Dicer or Dicer-Like enzymes), resulting in small interfering RNA (siRNAs) molecules of ~ 21 nucleotides. SiRNAs are loaded in the protein complex RISC (RNA-Induced Silencing Complex) but only the complementary strand to the target mRNA remains associated and guides its recognition and cleavage. An essential component of RISC is the Argonaute (Ago) protein, which is responsible of cutting the target mRNA [29].

In plants, in order to be efficient, RNA silencing is amplified in recipient cells by host RdRPs that make viral dsRNAs which are spread through plasmodesmata and the vascular system [28].

In mosaic leaves ToMV as well as ToMV siRNAs are present predominantly in the chlorotic areas but in few quantities in the green ones. Antiviral RNA silencing activity is registered mainly in the boundary of both regions in the "true dark green tissues". It has been suggested that this distribution restricts the expansion of the chlorotic areas where ToMV predominates and RNA silencing is overcome [30,31]. It is tough that the dsRNA in tomato is formed only after the PMTC is established in order to protect the structure and avoid viral RNA silencing [14]. ToMV avoids RNA silencing also by viral RNA silencing suppressor molecules, one of them is the soluble 130 KDa protein that possibly acts after siRNA production [30].

4. CONCLUSION

Infection of tomato crops with ToMV causes great economic losses worldwide. There have been established tomato strain resistant to the virus and the mechanisms underlying the resistance of the plant have begun to be described. The Hypersensitive Response and the Antiviral RNA Silencing mechanisms are until now described as the major contributors of immunity against the ToMV infection. However, more experiments are needed in order to better describe them. In the case of the Hypersensitive Response, apart of *Tm-1*, *Tm-2* and *Tm-2²*, other R proteins might be implied. Regarding Antiviral RNA Silencing, knockdown of suppressor viral molecules in crops might function to counteract the infection.

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