

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

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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. Some insights for ToMV control are discussed.

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

1. INTRODUCTION

The tomato plant (*Lycopersicon esculentum*) is a plant 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

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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 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]. ToMV does not have an invertebrate vector [7]; it is seed-borne; present mainly in the seed coat and the endosperm. Seeds come from infected plants but also it has been proposed that ToMV might enter the seed by the micropyle when is in contact with contaminated soils where virus stays for several years. Moreover, viral infection is transmitted mechanically through wounds in the plant caused by contaminated tools during manual practices such as transplantation or pruning [8,9,10]. Once inside the cell viral transcription of replication proteins is carried out, latter the movement protein helps the viral RNA to spread through plasmodesmata [11].



Fig. 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 (Figure 2) [12,13].

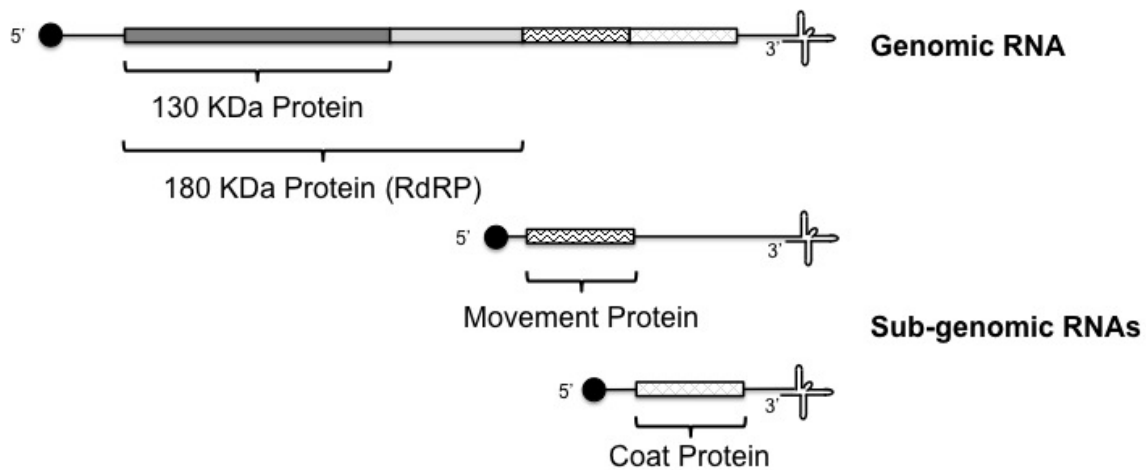


Fig. 2. ToMV RNA structure. Viral genome is 6.3 kbp with four open reading frames coding for the 130- and the read-through 180 kDa protein with activity of an RNA-dependent RNA Polymerase (RdRP). After replication, sub-genomic RNAs are synthesized and translated into the movement and coated protein, respectively. The 5' has a methylated cap and the 3' forms a tRNA-like structure [14].

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 [15,16].

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 [15]. 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 [14]. 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 [17].

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 [18]. In the case of Tobacco Mosaic Virus (TMV) -other Tobamovirus- replication complexes are formed on the endoplasmic reticulum membrane [17]. 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 [18,19].

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. These proteins bind to the

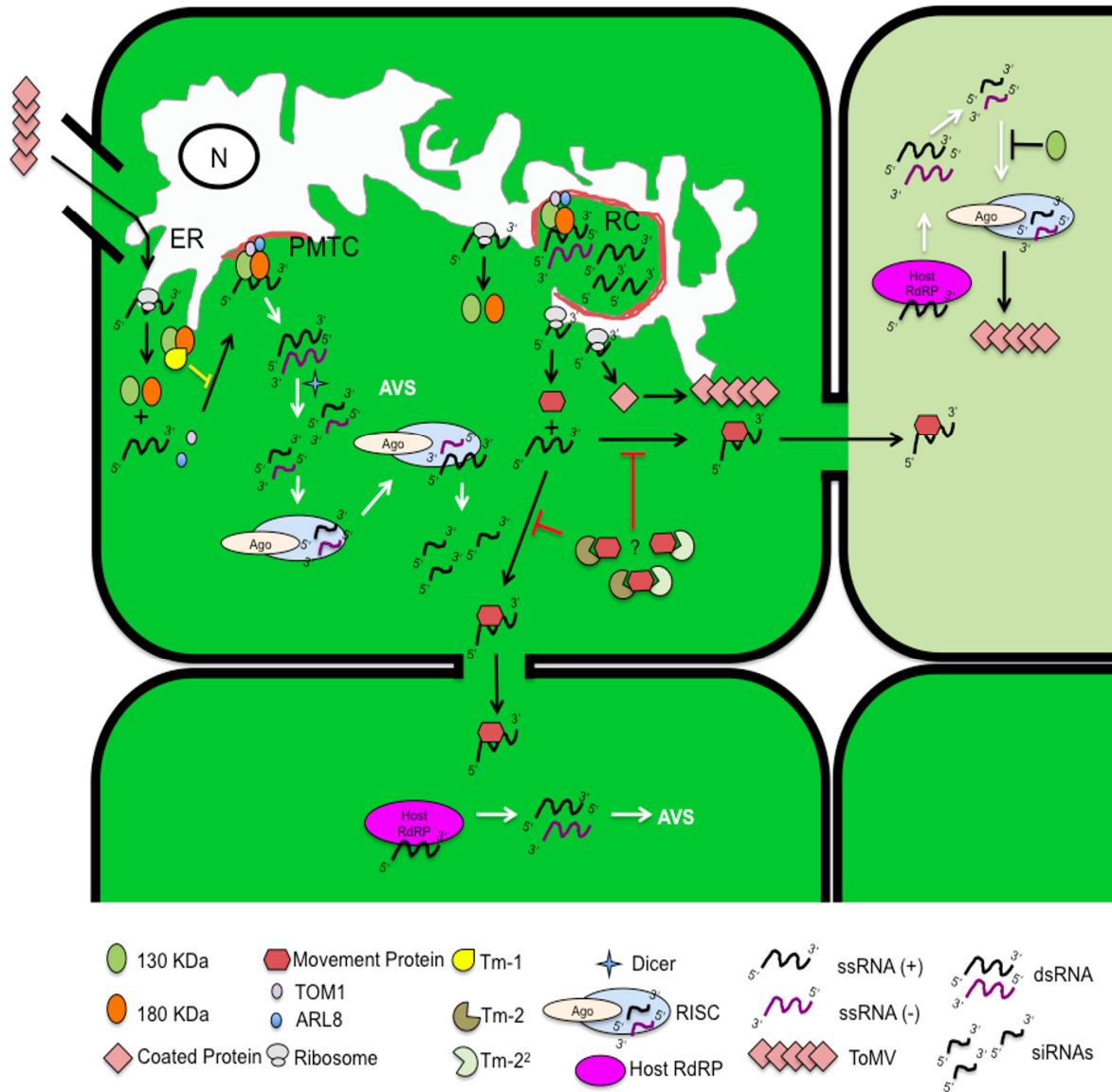
99 genomic viral 5' UTR giving rise to a pre-membrane-targeting complex (PMTC). The PMTC
100 is able to bind to membranes surfaces through the replication proteins, which in turn join to
101 host proteins TOM1 and ARL8 in order to initiate the synthesis of the negative strand
102 [15,20]. It is thought that RNA synthesis begins until the template RNA is isolated from
103 cytoplasm in order to avoid the double stranded RNA recognition produced during replication
104 by the host silencing machinery that would destroy it. In agreement with this, it seems that
105 the RdRP activity of replication proteins is acquired until them are associated to the
106 membrane [18].

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108 **3. IMMUNE RESPONSE IN *Lycopersicon esculentum***

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110 Growing under different environmental conditions *L. esculentum* is exposed to infectious
111 agents such as the ToMV but is able to trigger an immune response in order to counteract
112 the infection. Different from animals, plants do not possess an immune system based on
113 mobile cells, instead they trigger a molecular signal immunity activated by pattern-
114 recognition receptors (PRRs), by resistance proteins (R), and the RNA silencing machinery.
115 The plant-virus interaction elicits mainly the last two (Figure 2).
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117

118 **Fig. 3. Immune Response of *L. esculentum* against ToMV.** ToMV enter the cell through
 119 wounds. Immediately, replication proteins (130- and 180- kDa) are translated by host
 120 ribosomes and bind to viral genome and host proteins (TOM1 and ARL8) in order to
 121 form a pre-membrane targeting complex (PMTC). Once the replication complex (RC)
 122 has been established genomic and sub-genomic viral RNAs are synthesized and
 123 translated. Movement protein interacts whit the viral RNA forming a ribonucleoprotein
 124 particle that cross plasmodesmata spreading the infection to neighboring cells (black

arrows). Formation of the PMTC is inhibited by the interaction of Tm-1 with the replication proteins (truncated yellow line). Resistance proteins Tm-2 and Tm-2² bind the movement protein. Tm-2 binds to the N-terminal region, and Tm-2² to the C-terminal domain therefore inhibit the formation of the ribonucleoprotein particle and the spread of the viral RNA (indicated with truncated red lines). Is unknown if both proteins binds at the same time to the MP (indicated with the symbol “?”). These proteins conduct to a hypersensitivity response accompanied by programmed cell death. If viral RdRP activity is initiated before formation of the RC (white arrows), synthesis of viral dsRNAs are produced and are prone to be processed by the antiviral RNA silencing (AVS) machinery. Spread of AVS is through plasmodesmata and host RdRPs mediates its amplification. In some cases suppressor viral molecules such as the 130 kDa overcome AVS, this event is associated with chlorotic areas (yellow-green color in the figure) while non-infected tissues are green. ER: Endoplasmic Reticulum. N: nucleus.

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140 **3.1 Immunity Activated by Pattern-Recognition Receptors**

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142 Pathogen or Microbial Associated Molecular Patters (PAMPs or MAMPs) are conserved
143 structural elements of pathogenic and non-pathogenic microorganisms such as the
144 peptidoglycan structure present in the bacterial cell wall, the lipopolysaccharides in Gram-
145 negative bacteria, and oligosaccharides as well as chitin and its derivatives from de cell wall
146 of fungi. When PAMPs/MAMPs are recognized by Pattern-Recognition Receptors (PRRs)
147 the first line of defense in plants called MAMP-triggered immunity (MTI) is activated. Until
148 now viral MAMPs have not been described [6,21].

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150 **3.2 The Guard Hypothesis**

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152 As a second line of defense, a plant elicits the Effector-Triggered Immunity (ETI) in which
153 receptors known as resistance (R) proteins plays a pivotal role [21,22]. According to the
154 “guard” hypothesis, R proteins are activated when a self-protein is altered by an effector
155 pathogen molecule (the “avirulence” signal) [18]. In response to the avirulence signal plants
156 trigger a hypersensitive response (HR) frequently associated with programmed cell death at
157 the site of invasion resulting in necrotic lesions, as well as the production of antimicrobial
158 molecules. In some cases the HR is not activated [23,24].

159

160 R proteins include members of the NB-ARC-LRR family (Nucleotide Binding-ARC/Leucine-
161 Rich Repeat) [6,21]. They consist of the effector, the STAND and the LRR domains. The
162 effector domain in the N-terminal region is characterized by the presence of protein
163 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 of the pathogen sensing [25,26,27].

During ToMV infection *L. esculentum* switch 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 [28,29]. 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 [30,31]. 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 they activates the ETI response, it might be possible that they trigger different mechanisms of resistance. According with this, Tm-2²-MP interaction is more sensitive when amino acid changes are present in the latter compared with Tm-2-MP interaction [30,32].

3.2.2 Tm-1

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 semi-dominant gene that is formed by nine exons and eight 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 ToMV replication [28].

Tm-1 cellular role is unknown, but it inhibits ToMV replication. 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 [19,20,28].

3.3 Antiviral RNA Silencing

The RNA silencing mechanism controls gene expression by means of the endonucleolytic cleavage of transcripts or their translation inhibition [33]. 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 [34].

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 [23,33].

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 [35,36]. 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 [18]. 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 [35].

4. CURRENT INSIGHTS FOR CONTROL OF TOBAMOVIRUS

Several efforts have been reported for control of TMV infections, such as the generation of resistant varieties of tomato, transgenic tomato lines against virus as well as the induction of systemic resistance in plants. Direct chemical control of virus has been poorly explored, however, there are reports about the use of biomolecules that induce defense against Tobamovirus or have direct anti-viral activity, maybe affecting the replication rate of virus in the plant. For example, whey proteins such as α -lactalbumin, β -lactalbumin and lactoferrin have showed anti-viral effect in TMV infections when are esterified in order to preserve the proteins positive charges and avoid fast degradation. In Tobacco plants (*Nicotiana tabacum* var. samsun NN) they increase the defense related response and reduce the number of lesions caused by TMV since induce the over production of the defense enzymes ascorbate peroxidase (AP), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione peroxidase (GP), guaiacol-dependent peroxidases (GPOX) and defense-related enzymes phenylalanine ammonia lyase (PAL), and lipoxygenase (LOX) [37].

Polysaccharides as lentinan stimulated the defense related response in tobacco plants against TMV by the increase in the activity of PAL enzyme and phenylpropanoid compounds [38]. Peptaibols secreted by some strains of *Pseudomonas chlororaphis* O6 has showed activity against TMV [39]. Gossypol and β -sitosterol extracted from cotton have shown protective and curative effects in tobacco plants infected with TMV but also in rice plants infected with Rice stripe virus, a non-tobamovirus, indicating the inespecific anti-viral activity [40]. Derivatives of ferulic acid have shown anti-viral activity against TMV, as well as, some 1,2,3 thiadizole derivatives showed inactivation of virus and protection before infection [41].

Therefore, this related-defense activation could help to priming the specific R genes or other defense response in tomato against TMV and ToMV infections.

5. CONCLUSION

Infection of tomato crops with ToMV causes great economic losses worldwide. There have been established tomato strains 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. Notably, biomolecules stimulating immune response against viruses could help to counteract ToMV infection. Importantly these treatments might be environmentally friendly.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

MFMS, TLEY, GCKY, SHV, GUMD, MFJS managed the literature searches and discussed the information presented. MSMF and MFJS wrote the paper. All authors read and approved the final manuscript.

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