International Journal of Biochemistry Research & Review

19(4): 1-8, 2017; Article no.IJBCRR.37065
ISSN: 2231-086X, NLM ID: 101654445

Immune Response of *Lycopersicon esculentum* against the *Tomato mosaic virus*

Mendoza-Figueroa María Saraí[1*], Torres-López Elisa Yoalli[1], García-Cardoso Karina Yunuen[1], Solanes-Herrera Valeria[1], Gómez-Ulloa Milos David[1] and Mendoza-Figueroa José Silvestre[2]

[1]Departamento de Atención a la Salud, Universidad Autónoma Metropolitana, 04960, Mexico City, México.
[2]Departamento de Química de Biomacromoléculas, Instituto de Química, Universidad Nacional Autónoma de México, 04510, Mexico City, México.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MFMS, TLEY, GCKY, SHV, GUMD and MFJS managed the literature searches and discussed the information presented. Authors MFMS and MFJS wrote the paper. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2017/37065
Editor(s):
(1) Muhammad Abdul Rehman Rashid, Assistant Professor, Department of Plant Breeding and Genetics at "University of Agriculture Faisalabad", Pakistan.
Reviewers:
(1) Rafael Fernandez Da Silva, University of Carabobo, Venezuela.
(2) Davi Coe Torres, Universidade Federal de Alfenas, Brasil.
Complete Peer review History: http://www.sciencedomain.org/review-history/21695

Mini-review Article

Received 29th September 2017
Accepted 27th October 2017
Published 2nd November 2017

ABSTRACT

The tomato plant (*Lycopersicon esculentum*) is a crop rich in nutriments 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-2^2, as well as the Antiviral RNA Silencing. Some insights for ToMV control are discussed.

Keywords: ToMV; mosaic; R proteins; antiviral RNA silencing.

**Corresponding author: E-mail: msmendozafigueroa@gmail.com;*

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 has beneficial effects on blood pressure and apolipoprotein apoA-I levels reducing cardiovascular risk [3]. Thus the tomato plant represent an important crop in agriculture, representing the second most important crop at worldwide production with 100 million tons of fresh fruit pear year [4].

Tomato is cultivated in different geographical areas exposing this to different environmental conditions. That situation involves to the crops being susceptible to develop diseases caused by viruses, bacteria and fungi, advantaging the poor quality of product and subsequently high monetary losses. In this review we will summarize the immune response that *L. esculentum* triggers to counteract the *Tomato mosaic virus* (ToMV) infection, the etiological agent of one of the principal diseases that affects tomato crops.

2. *Tomato mosaic virus*

The ToMV is a pathogen that infects mainly tomato plants in a systemically way, causing mosaic symptoms, they are described like alternation of leaf color given chlorotic and dark green areas through leaves surface, as well as leaf curling (Fig. 1) [5,6]. ToMV does not have an invertebrate vector [7]; it is seed-borne transmitted virus; present mainly in the seed coat and the endosperm. In Infected seeds from viruliferous plants has been proposed that ToMV can be enter to the seed by the micropyle when is in contact with contaminated soils where virus persists 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 viral particles cross the cells barriers, viral transcription of replication proteins is carried out; latter the movement protein helps the viral RNA to spread through plasmodesmata [11].

The ToMV belongs to the genus Tobamovirus of the family Virgaviridae. It has a single-stranded, positive-sense, RNA genome [5] with around 6.3 Kbp. It has four open reading frames (ORFs) coding for the 130- and 180-kDa replication proteins, the movement protein, and the coat protein (Fig. 2) [12,13].

Fig. 1. *Lycopersicon esculentum* infected with ToMV. Chlorotic and green areas disposed in "mosaic" are appreciated as well leaf curling

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 has 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 [14,15,16].

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 has shown in *in vitro* experiments interaction with single stranded nucleic acids giving rise to a ribonucleoprotein that has been proposed to facilitate the intercellular passage through plasmodesmata to disperse the infection to new host cells [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, besides, the CP plays a dispensable role in the establishment of the virus replication complex [17].

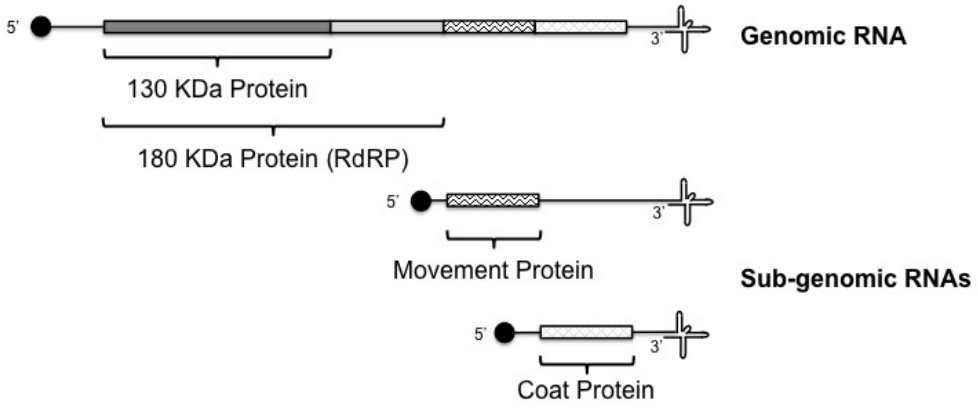

Fig. 2. ToMV RNA structure. Viral genome is 6.3 Kbp with four open reading frames coding for the 130- and the read-through 180 kDa protein with activity of an RNA-dependent RNA Polymerase (RdRP). After replication, sub-genomic RNAs are synthesized and translated into the movement and coated protein,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

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].

Once virions are in the cytoplasm, the translation of ToMV RNA is achieved, and the proteins 130- and 180-KDa are synthesized. During the next translation steps, these proteins bind to the genomic viral 5' UTR giving rise to a pre-membrane-targeting complex (PMTC). The PMTC can binds to the surface of membranes through the replication proteins, which in turn, binds to the host proteins TOM1 and ARL8, with the aim to initiate the synthesis of the negative RNA strand [15,20]. It has been thought that RNA synthesis begins until the template RNA is isolated from cytoplasm to avoid the recognition 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 them are associated to the membrane [18].

3. IMMUNE RESPONSE IN *Lycopersicon esculentum*

Growing under different environmental conditions *L. esculentum* is exposed to different infectious agents such ToMV, but the plant can trigger an immune response to counteract the infection. Different from animals, plants do not possess an immune system based on mobile cells, instead, they trigger immune molecular signals activated by pattern-recognition receptors (PRRs), resistance proteins (R), and the RNA silencing machinery. The plant-virus interaction elicits mainly the last two pathways (Fig. 3).

3.1 Immunity Activated by PAMPS

Pathogen Associated Molecular Patters (PAMPs) are conserved structural elements of pathogenic microorganisms, such as, peptidoglycan 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 fungus; also capsid proteins of virus are considered as PAMPs. When PAMPs are recognized by Patterns Recognition Receptors (PRRs) the first line of defense in plants called PAMP-triggered immunity (PTI) is activated [6,21].

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 [21,22]. 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 [23,24].

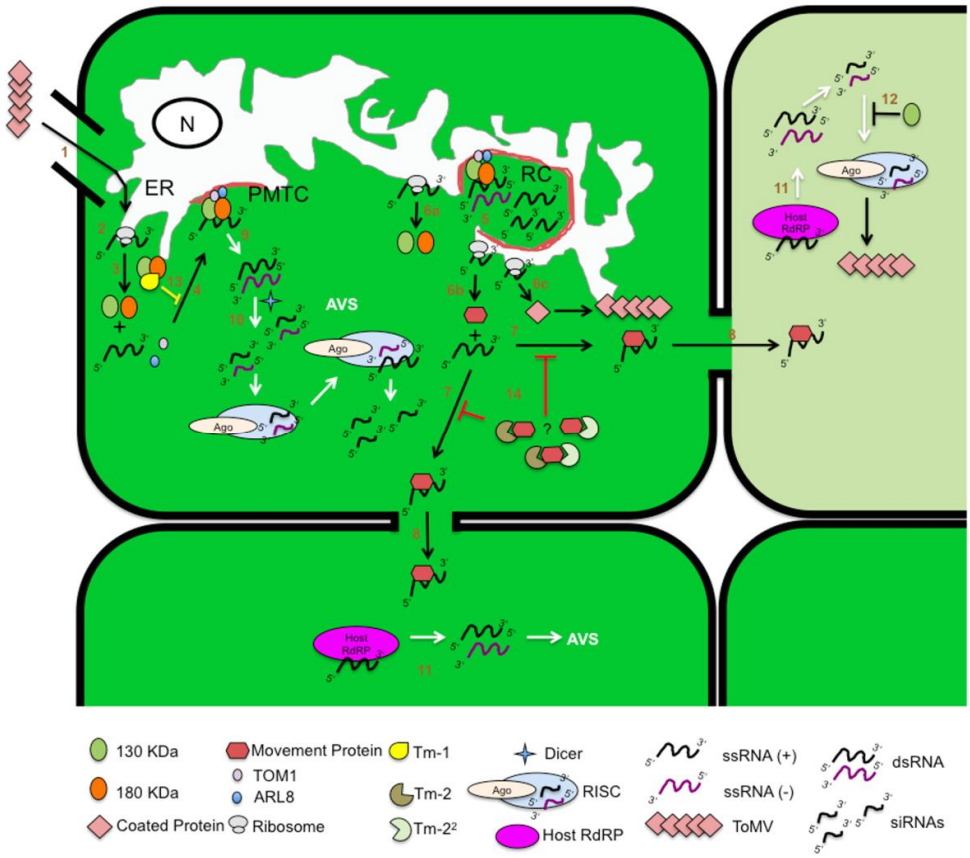

Fig. 3. Immune Response of *L. esculentum* against ToMV. ToMV enters the cell through wounds (1). Immediately, replication proteins (130- and 180- kDa) are translated by host ribosomes (2) and bind to viral genome and host proteins TOM1 and ARL8 (3) in order to form a pre-membrane targeting complex (PMTC) (4). Once the replication complex (RC) has been established genomic and sub-genomic viral RNAs are synthesized (5) and translated (6a, 6b, 6c). The movement protein interacts with the viral RNA forming a ribonucleoprotein particle (7) that pass through plasmodesmata spreading the infection to neighboring cells (8). If viral RdRP activity is initiated before formation of the RC (white arrows), synthesis of viral dsRNAs are produced (9) and are prone to be processed by the antiviral RNA silencing (AVS) machinery (10). Spread of AVS is through plasmodesmata and host RdRPs mediates its amplification (11). In some cases suppressor viral molecules such as the 130 kDa overcome AVS (12), this event is associated with chlorotic areas (yellow-green color in the figure) while non-infected tissues are green. Formation of the PMTC is inhibited by the interaction of Tm-1 with the replication proteins (truncated yellow line; 13). Resistance proteins Tm-2 and Tm-2^2 bind the movement protein (14). Tm-2 binds to the N-terminal region, and Tm-2^2 to the C-terminal domain therefore inhibit the formation of the ribonucleoprotein particle (indicated with truncated red lines) and the spread of the viral RNA. Is unknown if both proteins bind at the same time to the MP (indicated with the symbol "?"). These proteins conduct to a hypersensitivity response accompanied by programmed cell death. ER: Endoplasmic Reticulum. N: nucleus

R proteins include members of the NB-ARC-LRR family (Nucleotide Binding-ARC/Leucine-Rich Repeat) [6,21]. They have three domains: the effector, the STAND and the LRR. The effector domain in the N-terminal region is characterized by the presence of protein interacting regions 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-2^2, but also through other class of resistance proteins such as Tm-1 [28,29]. Genes coding for these proteins have been introduced in cultivated tomato species to control ToMV infection.

3.2.1 Tm-2 and Tm-2^2

Tm-2 and Tm-2^2 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-2^2 recognizes the C-terminal region. Both proteins trigger ETI response but possibly by different mechanisms. According with this, Tm2^2-MP interaction is more sensitive when amino acid changes are present in the MP compared with Tm-2-MP interaction [30,32].

3.2.2 Tm-1

The Tm-1 protein does not belong to the NB-ARC-LRR protein family; 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 inhibiting 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 through the endonucleolytic cleavage of transcripts or their translation inhibition [33]. The degradation of double stranded RNA (dsRNA) structure stars 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 guiding the mRNA recognition and cleavage. An essential component of RISC is he Argonaute (Ago) protein, which is responsible of cutting the target mRNA [34].

In plants, in order to be efficient, RNA silencing is amplified in host cells by host RdRPs that make viral dsRNAs, which are spread through plasmodesmata and the vascular system [23,33].

In symptomatic leaves with mosaic infected with 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]. ToMV avoids RNA silencing also by viral RNA silencing suppressor molecules, one of them is the soluble130 kDa protein that possibly acts after siRNA production [35].

4. CURRENT INSIGHTS FOR CONTROL OF TOBAMOVIRUS

Several efforts have been reported for controlling 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. The direct chemical control of virus has been poor explored, however, there are reports about the use of biomolecules that induce defense against tobamovirus or have direct anti-viral activity, might be affecting the replication rate of virus in the plant. For example, whey proteins such α-lactoalbumin, β-lactoalbumin 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 inducing 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 lipooxygenase (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 unspecific 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-2^2, 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.

ACKNOWLEDGEMENTS

TLEY, GCKY, SHV and GUMD are bachelor students of the Biology program at the Universidad Autónoma Metropolitana – Xochimilco. This work is the result of the Modular Investigation carried out at the Division of Biological and Health Sciences.

MFJS is a doctoral student from Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM) who kindly reviewed and assisted in the manuscript writing.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Beecher GR, Nutrient content of tomatoes and tomato products. Proc Soc Exp Biol Med. 1998;218(2):98-100.
2. Martí R, Roselló S, Cebolla-Cornejo J. Tomato as a source of carotenoids and polyphenols targeted to cancer prevention. Cancers (Basel). 2016;8(6):58.
   DOI: 10.3390/cancers8060058
3. Shidfar F, Froghifar N, Vafa M, Rajab A, Hosseini S, Shidfar S, Gohari M. The effects of tomato consumption on serum glucose, apolipoprotein B, apolipoprotein A-I, homocysteine and blood pressure in type 2 diabetic patients. Int J Food Sci Nutr. 2011;62(3):289-94.
   DOI: 10.3109/09637486.2010.529072.
4. Food and Agriculture Organization: Tomato; 2017.
   Available:http://www.fao.org/land-water/databases-and-software/crop-information/tomato/en/
5. Adams MJ, Antoniw JF, Kreuze J. Virgaviridae: A new family of rod-shaped plant viruses. Arch Virol. 2009;154(12): 1967-72.
   DOI: 10.1007/s00705-009-0506-6
6. Andolfo G, Ferriello F, Tardella L, Ferrarini A, Sigillo L, Frusciante L, Ercolano MR. Tomato genome-wide transcriptional

response to Fusarium wilt and *Tomato mosaic virus*. PLoS One. 2014;9(5): e94963.
DOI: 10.1371/journal.pone.0094963

7. Bachand GD, Castello JD. Seasonal pattern of Tomato Mosaic Tobamovirus infections and concentration in red spruce seedlings. Appl Environ Microbiol. 1998; 64(4):1436-41.
8. Sheoran IS, Olson DJ, Ross AR, Sawhney VK. Proteome analysis of embryo and endosperm from germinating tomato seeds. Proteomics. 2005;5(14):3752-64.
9. Kumar S, Udaya Shankar AC, Nakaya SC, Lund OS, Prakash HS. Detection of Tobacco mosaic virus and *Tomato mosaic virus* in pepper and tomato by multiplex RT-PCR. Lett Appl Microbiol. 2011;53(5): 359-63.
10. Gómez Jaimes R, Hernández Fuentes LM, Osuna García JA, Martínez Bolaños M. Virus fitopatógenos que afectan al cultivo de tomate en el estado de Nayarit. Folleto Técnico INIFAP-SAGARPA. 2013;25:28.
11. Ashby J, Boutant E, Seemanpillai M, Groner A, Sambade A, Ritzenthaler C, Heinlein M. Tobacco mosaic virus movement protein functions as a structural microtubule-associated protein. J Virol. 2006;80(17):8329-44. Erratum in: J Virol. 2006;80(24):12433. Groner, Anna [added].
12. Ohno T, Aoyagi M, Yamanashi Y, Saito H, Ikawa S, Meshi T, Okada Y. Nucleotide sequence of tobacco mosaic virus (tomato strain) genome and comparison with the common strain genome. J Biochem. 1984; 96(6):1915-23.
13. Fillmer K, Adkins S, Pongam P, D'Elia T. Complete Genome Sequence of *Tomato mosaic virus* Isolated from Jasmine in the United States. Genome Announc. 2015; 3(4).
pii: e00706-15.
DOI: 10.1128/genomeA.00706-15.
14. ViralZone. Tobamovirus.
Available:http://viralzone.expasy.org/51
15. Ishibashi K, Nishikiori M, Ishikawa M. Interactions between tobamovirus replication proteins and cellular factors: the impacts on virus multiplication. Mol Plant Microbe Interact. 2010;23(11):1413-9.
DOI: 10.1094/MPMI-04-10-0102.
16. Liu C, Nelson RS. The cell biology of Tobacco mosaic virus replication and movement. Front Plant Sci. 2013;4:12.
DOI: 10.3389/fpls.2013.00012
17. Asurmendi S, Berg RH, Koo JC, Beachy RN. Coat protein regulates formation of replication complexes during tobacco mosaic virus infection. Proc Natl Acad Sci U S A. 2004;101(5):1415-20.
18. Nishikiori M, Dohi K, Mori M, Meshi T, Naito S, Ishikawa M. Membrane bound *Tomato mosaic virus* replication prot eins participate in RNAsynthesis and are associated with host proteins in a pattern distinct from those that are not membrane bound. J Virol. 2006;80(17):8459-68.
19. Ishibashi K, Ishikawa M. The resistance protein Tm-1 inhibits formation of a *Tomato mosaic virus* replicationprotein-host membrane protein complex. J Virol. 2013;87(14):7933-9.
DOI: 10.1128/JVI.00743-13.
20. Ishibashi K, Ishikawa M. Mechanisms of *Tomato mosaic virus* RNA replication and its inhibition by the host resistance factor Tm-1. Curr Opin Virol. 2014;9:8-13.
DOI: 10.1016/j.coviro.2014.08.005
21. Newman MA, Sundelin T, Nielsen JT, Erbs G. MAMP (microbe-associated molecular pattern) triggered immunity in plants. Front Plant Sci. 2013;4:139.
DOI: 10.3389/fpls.2013.00139
22. Chaudhary R, Atamian HS. Resistance-gene-mediated defense responses against biotic stresses in the crop model plant tomato. J Plant Pathol Microbiol. 2017;8:4.
DOI: 10.4172/2157-7471.1000404
23. Boualem A, Dogimont C, Bendahmane A. The battle for survival between viruses and their host plantas. Curr Opin Virology. 2016;17:332-38.
24. Kobayashi M, Yamamoto-Katou A, Katou S, Hirai K, Meshi T, Ohashi Y, Mitsuhara I. Identification of an amino acid residue required for differential recognition of a viral movement protein by the *Tomato mosaic virus* resistance gene Tm-2^2. J Plant Physiol. 2011;168(10):1142-5.
DOI: 10.1016/j.jplph.2011.01.002
25. Van Ooijen G, Mayr G, Kasiem MMA, Albrecht M, Cornelissen BJC, Frank LW. Takken FLW. Structure–function analysis of the NB-ARC domain of plant disease resistance proteins. J Exp Bot. 2008;59(6): 1383-97.
DOI: 10.1093/jxb/ern045
26. Takken FLW, Tameling WIL. To nibble at plant resistance proteins. Science. 2009; 324(5928):744-6.
DOI: 10.1126/science.1171666

27. Urbach JM, Ausubel FM. The NBS-LRR architectures of plant R-proteins and metazoan NLRs evolved in independent events. Proc Natl Acad Sci USA. 2017; 114(5):1063-1068.
DOI: 10.1073/pnas.1619730114
28. Ishibashi K, Masuda K, Naito S, Meshi T, Ishikawa M. An inhibitor of viral RNA replication is encoded by a plant resistance gene. Proc Natl Acad Sci USA. 2007; 104(34):13833-8.
29. De Ronde D, Patrick Butterbach P, Kormelink R. Dominant resistance against plant viruses. Front Plant Sci. 2014;5:307.
DOI: 10.3389/fpls.2014.00307
30. Weber H, Ohnesorge S, Silber MV, Pfitzner AJP. The *Tomato mosaic virus* 30 kDa movement protein interacts differentially with the resistance genes *Tm-2* and *Tm-2*^{2}. Arch Virol. 2004;149(8): 1499-514.
31. Lanfermeijer FC, Jiang G, Ferwerda MA, Dijkhuis J, de Haan P, Yang R, Hille J. The durable resistance gene *Tm-2*^{2} from tomato confers resistance against ToMV in tobacco and preserves its viral specificity. Plant Sci. 2004;167(4):687-692.
32. Weber H, Pfitzner AJP. Tm-2^2 Resistance in tomato requires recognition of the carboxy terminus of the movement protein of *Tomato mosaic virus*. Mol Plant Microbe Interact. 1998;11(6):498-503.
33. Garcia-Ruiz H, Garcia Ruiz MT, Gabriel Peralta SM, Miravel Gabriel CV, El-Mounadi K. Mechanisms, applications, and perspectives of antiviral RNA silencing in plants. Rev Mex Fitopatol. 2016;34(3).
DOI: 10.18781/R.MEX.FIT.1606-8
34. Li Z, Rana TM. Molecular mechanisms of RNA-triggered gene silencing machineries. Acc Chem Res. 2012;45(7):1122-31.
DOI: 10.1021/ar200253u
35. Kubota K, Tsuda S, Tamai A, Meshi T. *Tomato mosaic virus* replication protein suppresses virus-targeted posttranscriptional gene silencing. J Virol. 2003;77(20): 11016-26.
36. Hirai K, Kubota K, Mochizuki T, Tsuda S, Meshi T. Antiviral RNA Silencing Is Restricted to the Marginal Region of the Dark Green Tissue in the Mosaic Leaves of *Tomato mosaic virus*-Infected Tobacco Plants. J Virol. 2008;82(7):3250-60.
DOI: 10.1128/JVI.02139-07
37. Wang J, Zhu YK, Wang HY, Zhang H, Wang HY. Inhibitory effects of esterified whey protein fractions by inducing chemical defense against tobacco mosaic virus (TMV) in tobacco seedlings, Ind. Crops Prod. 2012;37:207–212.
DOI: 10.1016/j.indcrop.2011.11.023
38. Wang J, Yu G, Li Y, Shen L, Qian Y, Yang J, Wang F, Inhibitory effects of sulfated lentinan with different degree of sulfation against tobacco mosaic virus (TMV) in tobacco seedlings, Pestic. Biochem. Physiol. 2015;122:38–43.
DOI: 10.1016/j.pestbp.2014.12.027.
39. Yeon Park J, Young Yang S, Kim YC, Le Dang O, Jun Kim J, Kim IS. Antiviral peptide from *Pseudomonas chlororaphis* O6 against tobacco mosaic virus (TMV). J Korean Soc Appli Biol Chem. 2012;55:89-94.
DOI: 10.1007/s13765-012-0015-2
40. Zhao L, Feng C, Hou C, Hu L, Wang Q, Wu Y. First discovery of acetone extract from cotton seed oil sludge as a novel antiviral agent against plant viruses. PLoS One. 2015;10:e0117496.
DOI: 10.1371/journal.pone.0117496
41. Huang GY, Cui C, Wang ZP, Li YQ, Xiong LX, Wang LZ. Synthesis and characteristics of (hydrogenated) ferulic acid derivatives as potential antiviral agents with insecticidal activity. Chem Cent J. 2013;7(1):33.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21695