EXPRESSION OF TOMATO PATHOGENESIS RELATED GENES IN RESPONSE TO *TOBACCO MOSAIC VIRUS*

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ABSTRACT

Tobacco mosaic virus (TMV) causes severe damages and economic losses of tomato crop production and quality worldwide. In the current study, the nucleotide sequence with NCBI-BLAST and phylogenetic analysis for the movement protein gene revealed that the Egyptian TMV isolate (Acc# MG264131) exhibited 99% identity with that of a Slovenian isolate (Acc# KY810785). Moreover, the transcription profiles of PR1, PR2, PR3, PR5 and PR7 genes in TMV-infected tomato tissues were analysed using semi-quantitative RT-PCR technique. Compared to mock-inoculated plants, the statistical analysis revealed significant changes in the relative expression level of both PR2 and PR7 at the two intervals 6 and 15 days post-inoculation with 32.3 and 34.7-fold change respectively. A significant change in transcription level of the two genes suggests that they play an essential role in the tomato defence system and could be disease-specific genes induced with the TMV infection. Based on the data obtained in this study and the previous studies, the down-regulation of PR2 and/or overexpression of PR7 in tomato plants could provide new insights in developing efficient strategies for reducing the tomato susceptibility to TMV infection.

Keywords: Tomato; TMV; Pathogen-related protein, RT-PCR.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops cultivated all over the world (Wang *et al.* 2018). The crop suffers moderate to severe losses due to different damages including abiotic and biotic stresses. Pathogens and pests are the major cause of crop deterioration causing different symptoms to the plant. Infection of the cultivated seedlings and/or mature plant can cause moderate to complete loss of the crop of the season; the magnitude of the infection depends on the time, location, environmental conditions as well as the causal agent (Barboza *et al.* 2018). *Tobacco mosaic virus* (TMV, genus *Tobamovirus*) causes systematic infection characterized by mosaic and\or chlorotic lesions on the leaves of infected plants, morphological alteration of the infected plants, and eventually complete loss of the plant productivity followed by plant death (Pfitzner *et al.* 2006; Abdelkhalek and Sanan-Mishra 2019). This disease can cause up to 100% crop loss as a result of blocking the development of reproductive organs, particularly flowering (Bazzini *et al.* 2007).

In order to achieve a successful systemic infection to the host plant, the virus establishes a mechanism to cell-to-cell transport and overcome the cellular barriers in the plant. Plant cell wall represents the first contact area that plays a crucial role in cellular signalling therefore, it is a target for different plant viruses (Otulak and Garbaczewska 2011; Otulak-Koziel *et al.* 2018). Callose acts as a physical barrier to the intracellular spreading of the viruses (Wu and Dimitman 1970; Ariizumi and Toriyama 2011).

Upon viral infection, plant starts serial changes in gene expression and the defence responses include the induction of numerous pathogenesis-related (PR) genes (Whitham *et al.* 2006; van Loon *et al.* 2006; Tabassum *et al.* 2013). PR genes play important roles in plant defence against biotic stresses and set genes of this group might be responsible for systemic acquired resistance against pathogens including plant viruses (van Loon *et al*. 2006; Elmorsi *et al*. 2015; Abdelkhalek *et al*. 2018). Hence, in this study, TMV was inoculated on tomato seedlings and the expression pattern of selected PR genes (PR1, PR2, PR3, PR5, and PR7) was studied at different time points of infection.

MATERIALS AND METHODS

Plant materials, viral infections, RNA extraction and cDNA synthesis: Infected tobacco plants exhibiting characteristic TMV symptoms were collected from the open fields at Alexandria government in Egypt. The collected samples were tested for TMV infection by DAS-ELISA as described by Clark and Adams (1977) and by using RT-PCR with specific primers (Table 1) as described previously by Jacobi *et al.* (1998). The TMV strain was maintained in *Nicotiana banthemiana* plants by mechanical inoculations. Under controlled conditions, Heinz 1706 tomato cultivar seeds were sterilized and

grown in vermiculite pots. At 35 days of seedlings, the true leaves were dusted with carborundum and gently inoculated with 1 ml of semi-purified TMV. Three independent biological replicates from mocked-inoculated and TMV-inoculated leaves were collected at 6, 9 and 15 days post-inoculation (dpi) and pooled for RNA isolations. Total RNA was extracted using Trizols (Invitrogen, CA, USA) according to the manufacturer procedure. A 1 µg of total RNA was used for cDNA synthesis using Superscript reverse transcriptase (Invitrogen, USA) according to manufacturer's instructions.

Semi-quantitative RT-PCR Analysis: Five primers (Table 1) specific to tomato PR genes (Kavroulakis *et al.* 2007) were used to check the expression levels. Actin gene was used as an internal control (Abdelkhalek and Sanan-Mishra 2019). Semi-quantitative RT-PCR reaction was carried out in 25 µl with an initial denaturation at 95°C for 2 min followed by 34 cycles at 95°C for 30s, 56°C for 30s, 72°C for 1 min and final extension step at 72°C for 5 min. A 10 µl of PCR products were by electrophoresed in 1.5% agarose gels and visualized under UV light after staining with ethidium bromide.

Sequencing analysis and phylogenetic construction: The PCR product was sequenced directly after purification with a PCR clean-up column kit (QIAGEN, Germany). Sequencing was performed using BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and model 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The obtained DNA nucleotide sequences were analysed using NCBI-BLAST for confirming the identity of the obtained sequences. The phylogenetic tree was tested with a bootstrap method with 2,000 replications and generated based on UPGMA statistical method using MEGA 5 software according to Tamura *et al*. (2011).

Statistical Analysis: The relative expression values of the three biological replicates for each time interval were statistically analyzed by one-way analysis of variance one-way (ANOVA) using the CoStat software and means were separated by least significant difference (LSD) test at $P \leq 0.05$ levels. The significant differences of the relative expression levels were plotted and standard deviation (± SD) is shown as a column bar. The values higher than 1 demonstrated an increase in the gene expression (up-regulation) while values lower than 1 means a decrease in expression levels (down-regulation).

RESULTS AND DISCUSSION

Molecular detection and characterization of TMV: TMV-inoculated tomato plants showed severe systemic mosaic with chlorosis symptoms at 15 days post-inoculation (dpi). The observed symptoms are identical to those reported by Cherian *et al*. (1999), Nassar *et al*. (2012) and Abdelkhalek and Sanan-Mishra (2019). By using RT-PCR, a 422 bp DNA fragment within the coding region of viral movement protein (MV) was amplified (Fig. 1). The amplified product was purified, sequenced and submitted to GenBank database under the accession number MG264131. NCBI-BLAST and phylogenetic analysis indicated that the TMV-Egyptian isolate is closely related to Slovenian (Acc# KY810785), USA (Acc# AF273221) and Finland (Acc# AF546184) isolates with a 99% identity (Fig. 2).

Response of PR genes to TMV infection: In order to clarify the molecular basis of the interaction between tomato plants and TMV, semi-quantitative RT-PCR technique was applied for the rapid identification of the changes of the expression level of PR1, PR2, PR3, PR5, PR7 genes in tomato during TMV infection (Fig. 3). By using TMV-MV gene specific primer, the inoculated plants gave positive results at 6, 9 and 15 dpi (Fig. 1). Comparing to mock-inoculated tomato and according to the one-way ANOVA analysis, the relative expression levels of some PR genes had significant differences at different time intervals (Fig. 4). Among these PR genes, the PR2 and PR7 were up-regulated with significant differences at 6 and 9 dpi for PR2 and 6, 9 and 15 dpi for PR7 (Fig. 3,4).

The gene expression profiling results revealed that PR1 was rapidly expressed in the mocked-treatment plants with 1-fold change (Fig. 4). Despite the expression was slightly down-regulation at 6 dpi with 0.9-fold change there was no statistically significant difference with mocked (Fig. 4). The continue decreasing of the relative expression to completely down-regulation at 15 dpi with 0.1-fold change (Fig. 4) suggesting that PR1 was not specifically associated with tomato-TMV infection. Thus, the expressed of PR1 in the mock plants might be the result of tomato wounding. As support for this hypothesis, the results of Chen *et al.* (2014) who observed that high expression of PR1 in mechanically wounded tomato with no significant expression in the unwounded plant. Although some results indicated that tobacco and tomato PR1 proteins have antiviral activity, the role of PR1 in plant defence remains poorly unclear (Cutt *et al*. 1989; Elmorsi *et al*. 2015). Many research groups have reported that overexpression of PR1 in transgenic plants resulted in increased resistance to oomycetes, fungi, bacteria, but not to viruses (Klessig *et al.* 2016; Breen *et al.* 2017).

Comparing to the mocked-treatment, PR2 gene showed significantly high transcriptions level at 6 dpi with 32.3-fold change (Fig. 3,4). Although the expression level was decreased to reach 6.6-fold change at 9 dpi there was a significant difference with mock treatment (Fig. 4). At 15 dpi (1.1-fold change), there was no statistically significant difference with mocked. The high

expression level of PR2 at 6 dpi suggesting that PR2 gene was associated with TMV infection. In addition, the decreasing of PR2 gene expression at 9 and 15 dpi could be explained by the silencing activity of TMV.

PR2 gene encodes a β-1,3-glucanases, plays a role in cell-cell spread of the virus movement complexes through limits callose accumulation near PD (Gillespie *et al.* 2002; Kawakami *et al.* 2004; Otulak and Garbaczewska 2011). It has been postulated that TMV increases the activity of tobacco PR2 genes to facilitate its movement (Iglesias and Meins 2000). The results are in agreement with the previous finding that reported a clear induction of PR2 during viral infections in tobacco (Linthorst *et al*. 1990; Rezzonico *et al.* 1998; Sindelarova and Sindelar 2005), *Arabidopsis* (Oide *et al.* 2013), onion (Elmorsi *et al.* 2015) and potato (Otulak-Koziel *et al.* 2018) plants.

Several studies have shown that callose deposition at PD leads to decreased cell-to-cell movement, whereas callose deposition inhibiting treatments lead to increased size-exclusion limits of PD (Beffa *et al.* 1996; Iglesias and Meins 2000; Lee *et al.* 2011). Mutant tobacco plants deficient for PR2 showing reducing PD size exclusion limit and increased callose deposition thus decreased in their susceptibility to virus infection (Otulak-Koziel *et al.* 2018). Moreover, overexpression of β-1,3 glucanase in PVY infection expedited virus spread between cells (Bucher *et al.* 2001; Dobnik *et al.* 2013).

PR3 exhibit chitinase activity that catalyses the hydrolysis of chitin (Xayphakatsa *et al.* 2008; Jannoey *et al.* 2017). In this study, PR3 showed a steady-state expression level at all time points post-infection (Fig. 3). Although there was slightly up-regulation with 1.3-fold change at 6, 9 and 15 dpi there was no statistically significant difference between mock and different intervals (Fig. 4). Like PR1, the expression in both mocked- and infected-plants suggesting that PR3 is not specifically tomato-TMV infection. At 10 dpi, it was reported that treatment of both *Brachypodium distachyon* and *Setaria viridis* with the same virus showed altered PR3 gene expression, where the expression level was highly down-regulated in the later and increased in the former (Mandadi and Scholthof 2012; Mandadi *et al.* 2014). Moreover, up- and down-regulation of PR3 gene were observed in different genotypes of the same plant species using the same virus (Andronic *et al.* 2015). Thus, the up- or down-regulation of PR3 may depend on the type of plant and the plant genotype.

PR5 gene exhibited slight induction only in mock-treated plants and was completely down-regulated at all dpi (Fig. 3). PR5 gene that encodes thaumatin-like proteins is found in cell vacuoles and showed antifungal activities (Li *et al.* 2015). Chen *et al*. (2010) reported that *Beet severe curly top virus* infection increased the expression level of PR5 in *Arabidopsis thaliana*. Moreover, up-regulation of PR5 gene in *Tobacco vein banding mosaic virus*-infected tobacco plants was observed (Yang *et al.* 2016). Thus, the complete down-regulation of PR5 gene in the infected tomato tissues could be explained as either it is not a target to TMV infection, or the virus suppressed its expression.

PR7, the most conspicuous PRs in tomato that encodes plant proteases, known to be an important component of defence responses proteins and involved in microbial cell wall dissolution (van Loon and van Strien 1999; Jorda *et al*. 2000; Roylawar *et al*. 2015). In this study, although PR7 showed slightly induction in the mock-treatment, the transcription levels increase dramatically at 6 dpi to reach 3.6-fold change (Fig. 3,4). The expression level continued increased to 11.1-fold change at 9 dpi reaching the maximum levels of 34.7-fold change at 15 dpi (Fig. 4). The increasing the expression of PR7 with increasing the time of infection indicated that PR7 has some roles in defence responses against TMV in tomato cells. The results agree with Roylawar *et al*. (2015) who reported up-regulation of tomato PR7 in response to pathogen infections. Nevertheless, further characterisation and functional analysis of PR7 will lead to a more comprehensive understanding of tomato-TMV interactions.

It can be concluded that tomato PR1, PR3 and PR5 may not function as antiviral factors against TMV infection. On the other hand, the overexpression of PR7 with silencing of PR2 genes could enhance host defence and decrease susceptibility to TMV disease.

Table 1. List of primer sequences of the polyphenol biosynthetic genes and the housekeeping gene (β-actin) used in real-time PCR.

Gene name	Direction	Sequence 5'.....3'
MV	Forward	GACCTGACAAAAATGGAGAAGATCT
	Reverse	GAAAGCGGACAGAAACCCGCTG
PR1	Forward	CCAAGACTATCTTGCGGTTC
	Reverse	GAACCTAAGCCACGATACCA
PR2	Forward	TATAGCCGTTGGAAACGAAG
	Reverse	TGATACTTTGGCCTCTGGTC
PR3	Forward	CAATTCGTTTCCAGGTTTTG
	Reverse	ACTTTCCGCTGCAGTATTTG
PR5	Forward	AATTGCAATTTTAATGGTGC
	Reverse	TAGCAGACCGTTTAAGATGC
PR7	Forward	AACTGCAGAACAAGTGAAGG
	Reverse	AACGTGATTGTAGCAACAGG
β-actin	Forward	ATGCCATTCTCCGTCTTGACTTG
	Reverse	GAGTTGTATGTAGTCTCGTGGATT

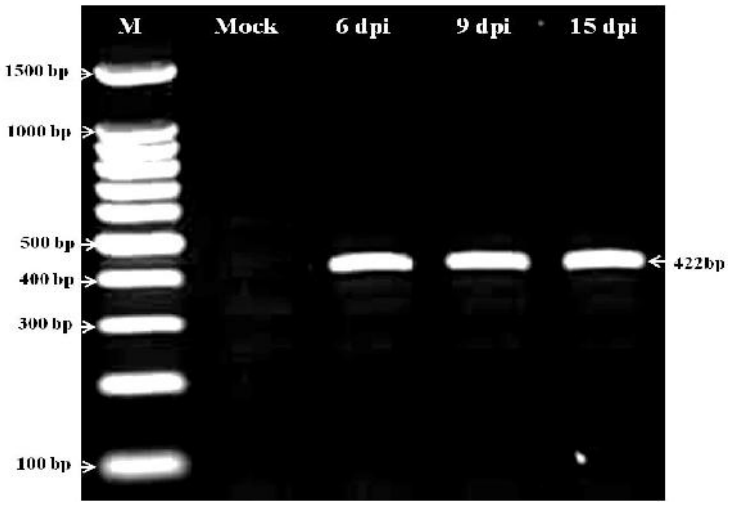

Figure 1. Agarose gel electrophoresis (1.5%) in TAE buffer stained with ethidium bromide showing RT-PCR of TMV-MV gene at 6, 9, and 15 day post inoculation (dpi) as well as mocked-inoculated plants. Lane M: 100 bp ladder DNA marker; Lane M: genetic pool for a mocked plant; Lanes 6 dpi, 9 dpi and 10 dpi: inoculated plants after 6, 9, and 15 days, respectively.

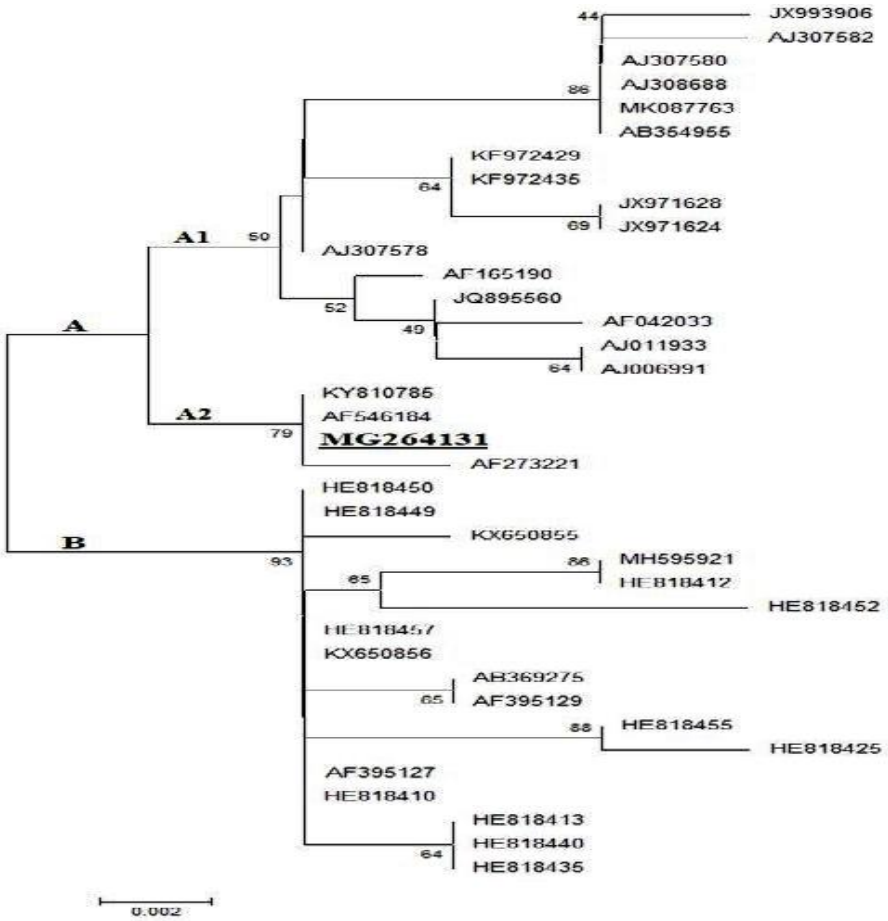

Figure 2. Phylogenetic analysis showing the genetic relationship between nucleotide sequence of the movement protein (MV) of the Egyptian TMV isolate (MG264131) and other MV genes of TMV isolates available in GenBank. The phylogeny tested with bootstrap method with 2,000 replications and generated based on UPGMA statistical method.

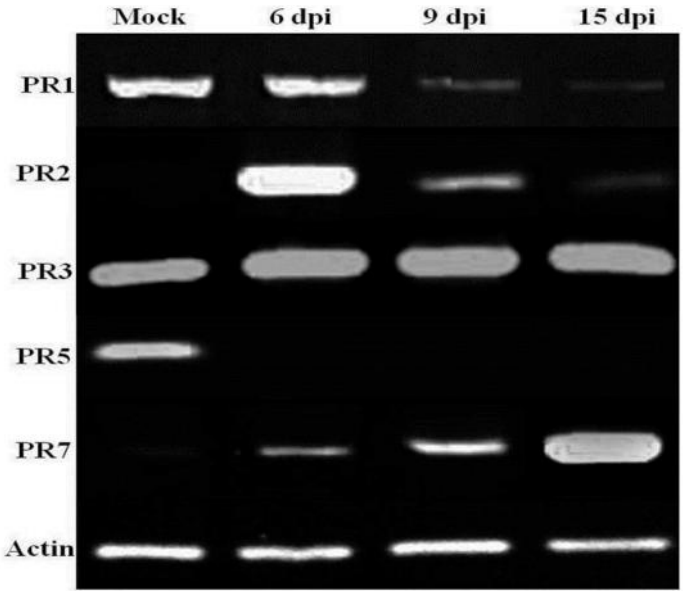

Figure 3. Agarose gel electrophoresis (1.5%) in TAE buffer stained with ethidium bromide showing RT-PCR for expression patterns of tomato PR genes (PR1, PR2, PR3, PR5 and PR7) upon TMV infection at 6, 9, and 15 dpi as well as mocked-treated plants. Actin gene used as reference gene.

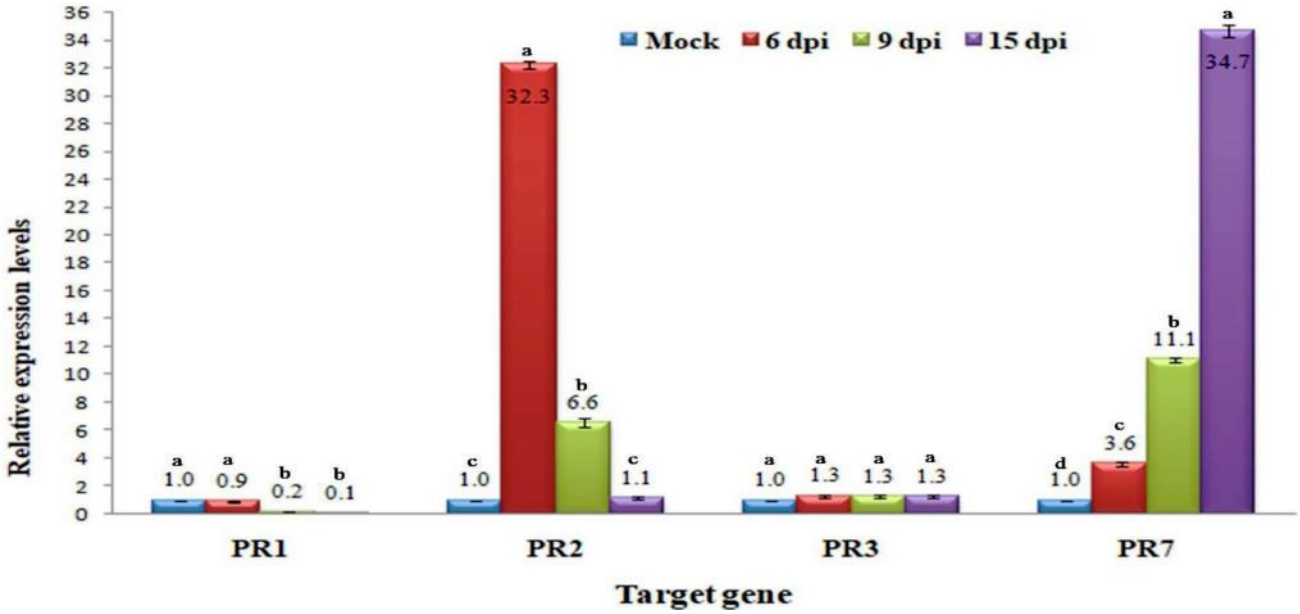

Figure 4. The relative expression levels of tomato PR genes upon TMV infection at 6, 9, and 15 dpi comparing to mocked tissues. The mocked sample was normalized to 1. Columns represent mean value from three biological replicates and bars indicate Standard Deviation (±SD). Significant differences between samples were determined by one-way ANOVA using CoStat software. Means were separated by Least Significant Difference (LSD) test at $P \leq 0.05$ levels and indicated by small letters. Columns with the same letter means do not differ significantly.

REFERENCES

Abdelkhalek, A. and N. Sanan-Mishra (2019) Differential Expression Profiles of Tomato miRNAs Induced by *Tobacco Mosaic Virus*. J. Agr. Sci. Tech. 21(2): 475-485.

Abdelkhalek, A., A. Elmorsi, O. Alshehaby, N. Sanan-Mishra, and E. Hafez (2018) Identification of genes differentially expressed in Iris yellow spot virus infected onion. Phytopathologia Mediterranea 57(2): 334-340.

Andronic, L., A. Port, and M. Duca (2015) Expression of some genes in barley under viral infection. J. ASM Life Sciences 2(326): 59-65.

Ariizumi, T. and K. Toriyama (2011) Genetic regulation of sporopollenin synthesis and pollen exine development. Annual Review of Plant Biology 62: 437-460.

Barboza, N., M. Blanco-Meneses, P. Esker, E. Moriones, and A.K. Inoue-Nagata (2018) Distribution and diversity of begomoviruses in tomato and sweet pepper plants in Costa Rica. Ann. Appl. Biol. 172: 20-32.

Bazzini, A.A., H.E. Hopp, R.N. Beachy, and S. Asurmendi (2007) Infection and coaccumulation of Tobacco mosaic virus proteins alter microRNA levels, correlating with symptom and plant development. Proc. Natl. Acad. Sci. 104: 12157-12162.

Beffa, R.S., R.M. Hofer, M. Thomas, and F.J.R. Meins (1996) Decreased Susceptibility to Viral Disease of [beta]-1,3-Glucanase-Deficient Plants Generated by Antisense Transformation. Plant Cell 8(6): 1001-1011.

Breen, S., S.J. Williams, M. Outram, B. Kobe, and P.S. Solomon (2017) Emerging insights into the functions of pathogenesis-related protein1. Trends Plant Sci. 10: 871-879.

Bucher, G.L., C. Tarina, M. Heinlein, F. Di Serio, F.J.R. Meins, and V.A. Iglesias (2001) Local expression of enzymatically active class I beta-1,3 glucanase enhances symptoms of TMV infection in tobacco. Plant J. 28: 361-69.

Chen, H., Z. Zhang, K. Teng, J. Lai, Y. Zhang, Y. Huang, Y. Li, L. Liang, Y. Wang, C. Chu, H. Guo, and Q. Xie (2010) Upregulation of LSB1/GDU3 affects geminivirus infection by activating the salicylic acid pathway. Plant J. 62: 12-23.

Chen, Y.L., C.Y. Lee, K.T. Cheng, W.H. Chang, R.N. Huang, H.G. Nam, and Y.R. Chen (2014) Quantitative peptidomics study reveals that a wound-induced peptide from PR-1 regulates immune signalling in tomato. Plant Cell 26: 4135-4148.

Cherian, S., J. Joseph, V. Muniyappa, and H.S. Savithri (1999) Characterization of tobacco mosaic virus isolated from tomato in India. Curr. Sci. 76: 1384-1388.

Clark, M.F., and A.N. Adams (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34(3): 475-483.

Cutt, J.R., M.H. Harpster, D.C. Dixon, J.P. Carr, P. Dunsmuir, and D.F. Klessig (1989) Disease response to tobacco mosaic virus in transgenic tobacco plants that constitutively express the pathogenesis-related PR1b gene. Virology 173: 89-97.

Dobnik, D., S. Baebler, P. Kogovsek, M. Pompe-Novak, D. Stebih, G. Panter, N. Janez, D. Morisset, J. Zel, and K. Gruden (2013) β-1,3-glucanase class III promotes spread of PVY^{NTN} and improves in planta protein production. Plant Biotechnol. Rep. 7: 547-555.

Elmorsi, A., A. Abdelkhalek, O. Alshehaby, and E. Hafez (2015) Pathogenesis-related genes as tools for discovering the response of onion defence system against Iris yellow spot virus infection. Botany 93(11): 735-744.

Gillespie, T., P. Boevink, S. Haupt, A.G. Roberts, R. Toth, T. Valentine, S. Chapman, and K.J. Oparka (2002) Movement protein reveals that microtubules are dispensable for the cell-to-cell movement of *Tobacco mosaic virus*. Plant Cell 14: 1207-1222.

Iglesias, V.A. and F.J.R. Meins (2000) Movement of plant viruses is delayed in a β-1,3-glucanase deficient mutant showing a reduced plasmodesmatal size exclusion limit and enhanced callose deposition. Plant J. 21(2): 157-166.

Jacobi, V., G.D. Bachand, R.C. Hamelin, and J.D. Castello (1998) Development of a multiplex immunocapture RT-PCR assay for detection and differentiation of tomato and tobacco mosaic tobamoviruses. J. Virological Methods 74: 167-178.

Jannoey, P., D. Channei, J. Kotcharerk, W. Pongprasert, and M. Nomura (2017) Expression analysis of genes related to rice resistance against brown planthopper, Nilaparvata lugens. Rice. Sci. 24: 163-172.

Jorda, L., V. Conejero, and P. Vera (2000) Characterization of P69E and P69F , two differentially regulated genes encoding new members of the subtilisin-like proteinasi family from tomato plants. Plant Physiology 122: 67-74.

Kavroulakis, N.S., G.I. Zervakis, C. Ehaliotis, K. Haralampidis, and K.K. Papadopoulou (2007) Role of ethylene in the protection of tomato plants against soil-borne fungal pathogens conferred by an endophytic Fusarium solani strain. J. Exp. Bot. 58: 3853-3864.

Kawakami, S., Y. Watanabe, and R.N. Beachy (2004) *Tobacco mosaic virus* infection spreads cell-to-cell as intact replication complexes. Proc. Natl. Acad. Sci. USA 101: 6291-6296.

Klessig, D.F., M. Tian, and H.W. Choi (2016) Multiple targets of salicylic acid and its derivatives in plants and animals. Front. Immunol. 7: 206.

Lee, J.Y., X. Wang, W. Cui, R. Sager, S. Modla, K. Czymmek, B. Zybaliov, K. Van Wijk, C. Zhang, H. Lu, and V. Lakshmanan (2011) A plasmodesmata-localized protein mediates crosstalk between cell-to-cell communication and innate immunity in Arabidopsis. Plant Cell 23: 3353-3373.

Li, X.Y., L. Gao, W.H. Zhang, J.K. Liu, Y.J. Zhang, H.Y. Wang, and D.Q. Liu (2015) Characteristic expression of wheat PR5 gene in response to infection by the leaf rust pathogen, Puccinia triticina. J. Plant Interact. 10(1): 132-141.

Linthorst, H.J., L.S. Melchers, A. Mayer, J.S. Van Roekel, B.J. Cornelissen, and J.F. Bol (1990) Analysis of gene families encoding acidic and basic beta-1,3-glucanases of tobacco. Proc. Natl. Acad. Sci. USA 87: 8756-8760.

Mandadi, K.K., and K.B.G. Scholthof (2012) Characterization of a viral synergism in the monocot Brachypodium distachyon reveals distinctly altered host molecular processes associated with disease. Plant Physiology 160: 1432-1452.

Mandadi, K.K., J.D. Pyle, and K.B. Scholthof (2014) Comparative analysis of antiviral responses in Brachypodium distachyon and Setaria viridis reveals conserved and unique outcomes among C3 and C4 plant defences. Mol. Plant Microbe. Interact. 27: 1277-1290.

Nassar, E.A., K.A. El-Dougdoug, M.E. Osman, R.A. Dawoud, and A.H. Kinawy (2012) Characterization and elimination of a TMV isolate infecting Chrysanthemum plants in Egypt. Int. J. Virol. 8: 14-26.

Oide, S., S. Bejai, J. Staal, N. Guan, M. Kaliff, and C. Dixelius (2013) A novel role of PR2 in abscisic acid (ABA) mediated, pathogen-induced callose deposition in *Arabidopsis thaliana.* New Phytol. 200: 1187-1199.

Otulak, K., and G. Garbaczewska (2011) Cellular localisation of calcium ions during potato hypersensitive response to Potato virus Y. Micron 42: 381-392.

Otulak-Koziel, K., E. Koziel, and B. Lockhart (2018) Plant Cell Wall Dynamics in Compatible and Incompatible Potato Response to Infection Caused by Potato Virus Y (PVY^{NTN}). Int. J. Mol. Sci. 19, 862.

Pfitzner, A.J.P. (2006) Resistance to Tobacco Mosaic Virus and Tomato Mosaic Virus in Tomato. In: Loebenstein G., Carr J.P. (eds) Natural Resistance Mechanisms of Plants to Viruses. Springer, Dordrecht.

Rezzonico, E., N. Flury, F.J.R. Meins, and R. Beffa (1998) Transcriptional down-regulation by abscisic acid of pathogenesis-related b-1,3-glucanase genes in tobacco cell cultures. Plant Physiology 117: 585-592.

Roylawar, P., S. Panda, and A. Kamble (2015) Comparative analysis of BABA and Piriformospora indica mediated priming of defence-related genes in tomato against early blight. Physiol. Mol. Plant Pathol. 91: 88-95.

Sindelarova, M., and L. Sindelar (2005) Isolation of Pathogenesis-Related Proteins from TMV-Infected Tobacco and their Influence on Infectivity of TMV. Plant Prot. Sci. 41: 52-57.

Tabassum, B., Z. Sher, M. Tariq, A. Khan, N. Shahid, M. Bilal, M. Ramzan, I.M. Shehzad, I.A. Nasir, and T. Husnain (2013) Overview of acquired virus resistance in transgenic plants. Exp. Agricult. Horticult. 2(2): 12-28.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, and M. Nei (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol. Biol. Evol. 28: 2731–2739.

Van Loon, L.C., and E.A. Van Strien (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol. Mol. Plant Pathol. 55: 85-97.

Van Loon, L.C., M. Rep, and C.M.J. Pieterse (2006) Significance of inducible defense-related proteins in infected plants. Annu. Rev. Phytopathol. 44: 135-162.

Wang, L., R. Zhao, R. Li, W. Yu, M. Yang, J. Sheng, and L. Shen (2018) Enhanced drought tolerance in tomato plants by overexpression of SlMAPK1. Plant Cell Tissue Organ Cult. 133: 27-38.

Whitham, S.A., C. Yang, and M.M. Goodin (2006) Global impact: elucidating plant responses to viral infection. Mol. Plant Microbe Interact. 19: 1207-1215.

Wu, J.H., and J.E. Dimitman (1970) Leaf structure and callose formation as determinants of TMV movement in bean leaves as revealed by UV irradiation experiments. Virology 40: 820-827.

Xayphakatsa, K., T. Tsukiyama, K. Inouye, Y. Okumoto, T. Nakazaki, and T. Tanisaka (2008) Gene cloning, expression, purification and characterization of rice (Oryza sativa L.) class II chitinase CHT11. Enzyme Microb Technol. 43(1): 19-24.

Yang, L., Y. Xu, Y. Liu, D. Meng, T. Jin, and X. Zhou (2016) HC-Pro viral suppressor from tobacco vein banding mosaic virus interferes with DNA methylation and activates the salicylic acid pathway. Virology 497: 244-250.