

DAMAGING EFFECTS OF TOMATO MOSAIC VIRUS AND POTATO VIRUS Y ON TOMATO PLANTS

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ABSTRACT

Tomato fruit is fairly high in vitamins A and C. It was regarded as a top priority vegetable by scientists interviewed under the FAO. Low tomato yields are due to a number of factors. Viral diseases have been ranked as one of the most important pathogens among tomato diseases. Four major viruses infect tomato in Bulgaria: *Tomato spotted wilt virus* (TSWV); *Tomato mosaic virus* (ToMV), *Cucumber mosaic virus* (CMV) and *Potato virus Y* (PVY). ToMV is a member of the genus *Tobamovirus*. It is distinguished from *Tobacco mosaic virus* (TMV) by its ability to produce local necrotic lesions in *Nicotiana tabacum* sp. Its symptoms include mosaic, systemic chlorosis, local necrotic lesions, leaf abscission, as well as systemic leaf and stem necrosis, which ultimately cause plant death. The virus is transmitted by human activities, through seed, and from leaf and root debris. ToMV causes great economic losses to farmers decreasing their fruit yield and reducing quality of tomato fruits.

Key words: *ToMV, PVY, tomato*

Introduction

The tomato (*Lycopersicon esculentum* Mill.) is a herbaceous fruiting plant. It originated in Latin America and has become one of the most widely grown vegetables with ability to survive in diverse environmental conditions (Rice *et al.*, 1987). Tomato fruit is considered to be fairly high in vitamins A and C, of high cash value and with potential for value-added processing. Tomato was regarded as a top priority vegetable by scientists interviewed under the Technical Advisory Committee of the Consultative Group on International Agricultural Research (CGIAR) (FAO, 1990). Recently, there has been more emphasis on tomato production not only as source of vitamins, but also as a source of income and food security. According to FAO (FAO, 1990, 2003) reports, tomato is now the most important vegetable in the tropics. It is annually planted on almost 4 million ha worldwide.

Low tomato yields are due to a number of factors. These include (1) lack of improved well-performing varieties; (2) poor fruit setting due to heavy rains and excessively high temperatures, which limit pollination, more specifically fecundation plus pollen viability; and (3) pests and diseases (Lyons, 1985).

About 146 viruses infect tomato worldwide (Green, 1991). They are grouped into 33 genera, but 15 genera are of the most economic importance, i.e. *Alfalfavirus*, *Begomovirus*, *Carlavirus*, *Crinivirus*, *Cucumovirus*, *Ilarvirus*, *Luteovirus*, *Nepovirus*, *Potexvirus*, *Potyvirus*, *Tobamovirus*, *Tombusvirus*, *Topocuvirus*, *Tospovirus*, and *Tymovirus*. These fifteen genera belong to families *Bromoviridae*, *Bunyaviridae*, *Closteroviridae*, *Flexiviridae*, *Geminiviridae*, *Luteoviridae* and *Potyviridae* (Pringle, 1999). Family *Bunyaviridae* has only one assigned plant-infecting genus (*Tospovirus*) to which *Tomato spotted wilt virus* (TSWV) belongs. Other genera of this family consist of virus species that infect animals only. Family *Flexiviridae* has been recently approved by ICTV (Mayo and Brunt, 2005). Its major tomato virus is *Potato virus X*, which belongs to genus *Potexvirus*. Major tomato viruses fall into five genera, i.e. *Tobamovirus*, *Cucumovirus*, *Tospovirus*, *Begomovirus*, and *Potyvirus* (Fauquet and Mayo, 1999; Brunt, 1990).

Tobacco mosaic virus (TMV) is the type species of the genus Tobamovirus. Another species of this genus is *Tomato mosaic virus* (ToMV), which is distinguished from TMV by its ability to produce local necrotic lesions in *Nicotiana tabacum* var. White Burley and *N. sylvestris* (Green and Kim, 1991). ToMV strains include those, which cause corky ring, crusty fruit, yellow streak and aucuba symptoms (Kang, 1981; Jones, 1991). Consequently, it is not easy to correctly identify ToMV by basing on symptoms because it causes a variety of them. However, known common ToMV symptoms include mosaic, systemic chlorosis, local necrotic lesions, leaf abscission, as well as systemic leaf and stem necrosis, which ultimately cause death (Brunt, 1990; Green and Kim, 1991). The virus is transmitted by human activities, through seed, and from leaf and root debris (Green and Kim, 1991). It is also readily sap-transmissible and cosmopolitan (Brunt, 1990). ToMV has been found as an aerosol in fog in USA (Castello, 1995) and in nutrient solution used for crop cultivation in Apulia, Italy (Pares, 1992; Gallitelli, 1982), and in Spain (Cordero, 1983). ToMV has been found in tomato cultivars with necrosis on the fruits (Dikova, 2013) and also in pepper plants in Bulgaria (Dikova, 2013). Potyviruses are the largest and economically most important group of plant viruses (Jones, 1991). In the VIDE database index of plant viruses (2006), for every 10 virus species listed at least one is a potyvirus. Potyviruses induce typical cylindrical, pinwheel-shaped inclusions in cells of infected plants (Green and Kim, 1991). Some major viruses in this family that infect tomato is *Potato virus Y* (PVY) (Zitter, 1974). Typical symptoms of PVY in tomato include mosaic, vein chlorosis, mild mottling, dark brown necrosis on leaflets, severe necrosis (Dikova, 2011), leaf crinkling, and drooping (Jones, 1991).

Material and methods

Tomato cultivars used: cv. Ideal, cv. Naslada, cv. Stryama, cv. Rila, cv. Kalina, cv. Buffalo heart, cv. Cherry, cv. Bononia, cv. Mila, cv. Heart of the Albeng.

DAS-ELISA The analysis was conducted by the method of Clark and Adams (1977). We used a commercial kit of LOEWE Biochemica GmbH, Sauerlach, Germany. ELISA plates were loaded with antiserum (IgG) for ToMV and respectively PVY, with dilutions (according to the instructions of the manufacturer) in 0.05 M carbonate buffer. The samples were incubated for 4 hours at 37 ° C, and the unbound components were washed out with PBS-T buffer for 5 min. All samples were grounded in extraction buffer containing 1% PVP (polyvinyl pyrrolidone) in a ratio of 1:10. The plates were incubated at 4 ° C for 16 hours. Following the third wash step alkaline-phosphatase conjugate for ToMV and respectively PVY was added and the plates were incubated for 4 hours at 37 ° C. The used substrate was p-nitrophenyl phosphate (p-nitrophenyl phosphate, Sigma) in diethanolamine buffer (pH 9.8) at a ratio of 1mg/ml. The reaction proceeded in the light at room temperature and was stopped with 3N NaOH. The adsorption of the color reaction was measured at multifunctional detector (DTX 880) at a wavelength of 405nm.

The positive samples had optical density (OD) over the threshold (Cut-off) which was two times the value of the negative control.

RNA extraction from potatoes infected with PVY: Extraction of total RNA was performed with RNEasy Plant Mini Kit (Qiagen, Germany). Extraction was carried out according to the instructions of the manufacturer.

Touch-Down RT-PCR

We used primers PVY Primer 1, 7 and 8 for P1 gene region of the virus, with program modification touch-down. Copy DNA synthesis: denaturation of total RNA (0,05-0,5 µg) at 95 ° C for 5 min with 10 µl PVY Primer1 primer in a final volume of 10 µl.; Cooling on ice to avoid renaturation; Preparation 15 µl of master mix: 5 µl of 5 × MMLV-buffer, 2 µl of dNTPs (2mM), 0.5 µl of M-MuLV Reverse transcriptase (200 U/µl), 7.5 µl H₂O. Incubation step at 42°C for 60 min. Master mix for the PCR is: 1 µl cDNA, 2.75 µl 10 × PCR buffer, 2.2 µl MgCl₂ (25 mM), 2.2 µl dNTPs (2 mM), 1 µl PVYPrimer1 (10 µM), 1 µl PVYPrimer7 (10 µM), 1 µl PVYPrimer8 (10

μl), 1 μl Taq DNA-Polymerase (5 U/μl), 12.85 μl H₂O. PCR was done in thermo cycler Auto-Q Server (LKB, UK) with following programme: initial denaturation step 3 min 95°C; five cycles 30 sec 92°C, 30 sec 62°C, 90 sec 72°C; five cycles 30 sec 92°C, 30 sec 60°C, 90 sec 72°C; five cycles 30 sec 92°C, 30 sec 58°C, 90 sec 72°C, ten cycles 30 sec 92°C, 30 sec 55°C, 90 sec 72°C; final elongation 10 min 72°C.

Results and discussion

Symptoms on the diseased plants:

ToMV symptoms were mosaic, systemic chlorosis, local necrotic lesions (Fig. 1 and Fig.2), leaf abscission, as well as systemic leaf and stem necrosis, which ultimately cause plant death. PVY symptoms were mainly mosaic chlorosis and distortion of the leaves (Fig.3) and mild necrosis on fruits.



Fig.1 ToMV necrosis



Fig.2 ToMV necrotic spots



Fig.3 PVY chlorosis

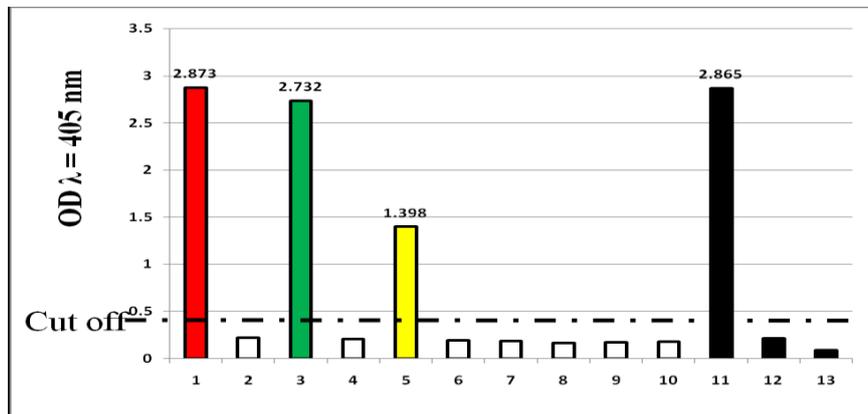


Fig.4 DAS-ELISA for ToMV from tomato cultivars

Only the tomato cultivars cv. Ideal, cv. Stryama, cv. Kalina showed virus infection of ToMV (Fig.4). Samples from other tested tomato cultivars were negative, under the Cut off value.

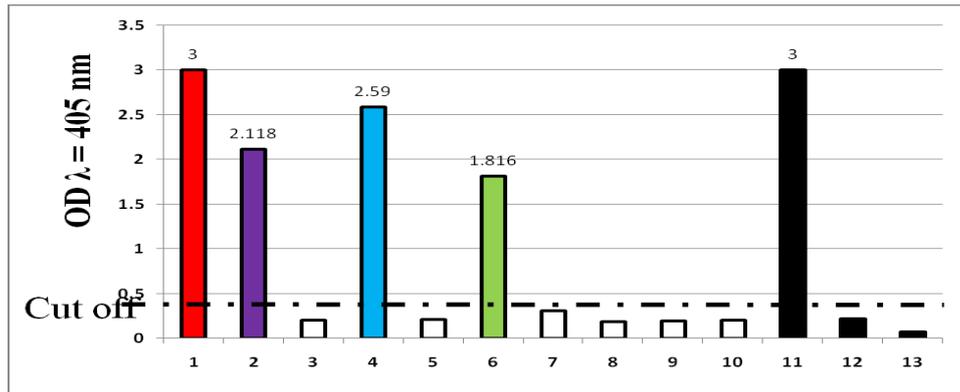


Fig.5 DAS-ELISA for PVY from tomato cultivars

The analyses done by DAS-ELISA showed that samples from tomato cv. Ideal, cv. Naslada, cv. Rila, cv. Buffalo heart were infected with PVY (Fig.5). The other samples for other tested tomato cultivars were negative.

For confirmation the results from DAS-ELISA for PVY we performed Touch down RT-PCR with specific primers for P1 gene region of the PVY genome. The results confirmed that tomato cultivars Naslada, cv. Rila, cv. Buffalo heart were infected with PVY^{N/NTN} group strain (Fig.6) We received a positive band of 443 bp product for these three tomato cultivars (Fig.6).

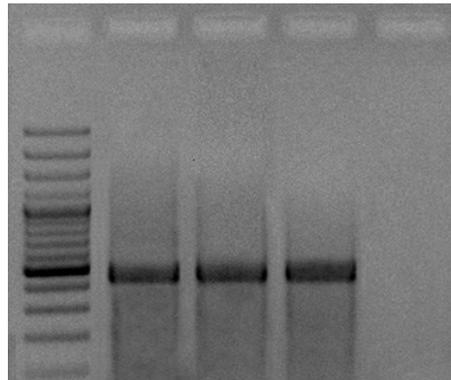


Fig. 6 Touch down RT-PCR for P1 region of PVY

Conclusion

ToMV is transmitted by human activities, through seed, and from leaf and root debris. It causes great economic losses to farmers decreasing their fruit yield and reducing quality of tomato fruits. PVY is transmitted mainly with aphids and together with ToMV causes damages to tomato plants and fruits. Only the cultivars Cherry, cv. Bononia, cv. Mila, cv. Heart of the Albeng remained virus free.

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References

1. Дикова, Б. 2013. Вирусна болест влошава пазарния вид на домати и пипер. *Земеделие плюс*, 4, 21
2. Дикова, Б. 2011. Тютюневият ратъл вирус. *Земеделие плюс*, 5, 34-36

3. Brunt, A., Grabtree K., and Gibbs A. 1990. Viruses of Tropical plants. CAB International. UK. 707 pp.
4. Castello, J. D., Lakshman, D. K., Tavantzis, S. M., Rogers, S. O., Bachand, G. D., Jagels, R., Carlisle, J., and Liu, Y. 1995. Detection of infectious *Tomato mosaic virus* in fog and clouds. *Phytopath.* 85: 1409 - 1412.
5. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme - linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.*, 34: 475-483.
6. Cordero, M., and Gaborjanyi, R. 1983. Study of interrelationship between TEV and TMV viruses in tomato plants. *Agrotecnia - de Cuba*, 15: 101 - 111.
7. FAO, 1990, Production Year Book 1989, Vol. 43.
8. FAO, 2003, World Agriculture Information Center Data base. Rome, Italy. http://www.fao.org/waicent/portal/statistics_en.asp.
9. Fauquet, M. C., and Mayo, M. A 1999. Abbreviations for plant virus names, 1999. *Arch. Virol.* 144:6.
10. Gallitelli, D., Savino, V., and Piazzolla, P 1982. Mixed infections of *Tobacco necrosis virus* and *Potato Virus Y* in tomato, Apulia, Italy. *Informatore - Fitopatologico*, 32: 43 - 45.
11. Green, S. K., and Kim J.S. 1991. Characterisation and control of viruses infecting peppers: a Literature Review. AVDRC, Tech. Bull. No. 18. 60 pp.
12. Green, S. K., and Kim J.S. 1991. Characterisation and control of viruses infecting peppers: a Literature Review. AVDRC, Tech. Bull. No. 18. 60 pp.
13. Jones, J. B., Stall, R. E., and Zitter, T. A. 1991. Compendium of Tomato Diseases. American Phytopathological Society. Library of Congress Catalog Card No. 91-71246. 73 pp.
14. Kang, K. Y.; Suh, J. K.; and Yu, I. C. 1981. Identification of viruses isolated from tomatoes and survey on the occurrence of virus diseases of tomatoes. *Horticulture and Sericulture*, 23: 10 - 17.
15. Lyons, J. M., and Howard Ferris. 1985. IPM for Tomatoes. University of California, Statewide IPM. Project Publication No. 3274.
16. Mayo, M. A., and Brunt, A. A. 2005. Plant Virus Taxonomy: Families, Genera, and Type Species of plant viruses and viroids, ICTV, 1 p.
17. Pares, R. D., Gunn, L. V., and Cresswell, G. C. 1992. *Tomato mosaic virus* infection in a recirculating nutrient solution. *J. Phytopath. (Germany F.R.)*, 135 (3), 192 - 198.
18. Pringle, C.R. 1999. Virus Taxonomy. The Universal system of virus taxonomy updated to include the new proposals ratified by the International Committee on Taxonomy of Viruses during 1998. *Arch. Virol.* 144: 2.
19. Rice, R. P., Rice, L. W., and Tindall, H. D., 1987. Fruit and Vegetable Production in Africa. Macmillan Publishers, U.K. 371 pp.
20. Zitter, T.A. 1974. Transmission of Pepper mottle virus from susceptible and resistant pepper cultivars. *Phytopath.* 65:110 – 114.