Detection of Tomato Mosaic Virus Viruses in Farms and Greenhouses and Breed Several Isolates of The Potato Virus Y From this Host

Iman abdel sattar abdel razzaq
College of Agriculture/Baghdad university, Plant protection Department
Plant diseases -Plant Viruses, Ministry of Higher Education & Scientific Research, Al-Furat Al-Awsat Technical University, Al-Rumaitha Technical Institute

Abstract

Tomato is one of the most important vegetables in Iran and in many countries. This study examines the spread of mosaic virus viruses in farms and greenhouses in the 2017-2018 crop year. Our study region is located in Yazd county in Iran. In this study, we collected 451 leaf samples with signs of mosaic. To identify the viruses, we used tests of DAS-ELISA, ACP-ELISA, specific polyclonal antibodies of Potato virus Y(PVY), Cucumber mosaic virus (CMV), Tomato mosaic virus (ToMV) and Arabis mosaic virus (ArMV) and Potivirus single-stranded antibody. Percentage of contamination of specimens collected with putty virus, CMV, PVY, ArMV and ToMV were 35.9%, 11.3%, 20.6%, 2.6% and 13.5%, respectively. Therefore, according to the results, PVY is the predominant virus among the collected samples. Four species of potato Y virus were selected to investigate the breed type of the collected PVY isolates. After extracting total RNA from the contaminated isolates, a fragment of 837 bp was amplified using a PVY-specific protease region of the P1 protease region. To identify the strains studied, in the RFLP test, PCR product was affected by the Hinc test. Then, the pair of specific primers of PVY strains were used. The results of the reaction showed that all four isolates were NTN race.

Keywords: *Tomato, Potato virus Y, Tomato mosaic virus, Arabis mosaic virus*

1. Introduction

The scientific name of the tomato plant is Lycopersicum esculentum Mill. This plant is one of the two cotyledons in the potato family. Viral diseases cause quantitative and qualitative damage to this plant. The essential viruses causing tomato mosaic in Iran are Potato virus Y (PVY), Cucumber mosaic virus (CMV), Tomato mosaic virus (ToMV), Arabis mosaic virus (ArMV) (Stare et al., 2015; Gao et al., 2016; Li et al., 2017; Azizan et al., 2017; Aseel et al., 2019). PVY is a member of the genus Potyvirus and from the Potyviridae family, and there is widespread global expansion. The virus was first reported in the world by Smith (1931a) on potatoes in the UK and Iran by Karimi (1967). The illness is 740 nanometers long and 11 nanometers wide (Shukla et al., 1991). The virus has a wide host range. Its genome consists of a single-stranded RNA fragment with positive polarity and has about 1000 nucleotides and only one open reading frame (ORF) (Chung et al., 2008; Valli et al., 2018).

It naturally infects more than nine families of plants, including potato, chicory, legume, spinach, some weeds and ornamental plants (Gulya et al., 2002; Otulak-Kozieł et al., 2018). In general, their races are divided into three tracks: O, N, C. New combinations between breeds N and O have emerged as a result of the presence of the two plants in the same plant. These races are called N: O races, the most famous of which are: NTN and Wilga. Zone 3 of the NTN race is similar to race O, and area 5 is identical to race N (Smith, 1931b).

2. Materials and method

2.1. Sampling and Detection of Mosaic Agent Viruses of tomato farms and greenhouses

This study examines the spread of mosaic viruses in farms and conservatories in the 2017-2018 crop year. We collected 161 and 290 samples with mosaic signs, respectively, from the greenhouse and farm of tomato cultivation areas of Yazd city (located in Iran). We used ACP-ELISA and AS-0573 tests to examine the contaminated specimens of the virus. We also used the DAS-ELISA test (Ozaslan et al., 2006; Ming et al., 2019) and multispecific antibodies to viruses produced at the German DSMZ

according to the instructions of Clark and Adams (1977) to detect PVY, CMV, ToMV, ArMV. The results were read by a 405 nm wavelength ELISA (made in America) machine half an hour after the last phase of the test. Specimens that had more than twice the mean absorbance of Salem samples were considered as virus-infected samples.

2.2. Biological purification and amplification of four PVY isolates

To determine race, four PVY isolates with the names T2, T5, G1, and G2 were selected. They were inoculated mechanically on the plants for the purpose of biological purification. Then, the localized patches were transferred to the tomato plants for propagation. For inoculation 0.05 M phosphate buffer, pH seven and carborandum powder were used. Infected bushes with amplified isolates, were kept in greenhouse conditions at temperatures of 25 to 30 degrees Celsius.

2.3. Total RNA extraction and PCR-RT reaction

Total RNA was extracted from the Rneasy Plant Mini Kit (manufactured by Kiaagen Company, Germany) based on the company operating instructions. Quantitative and quantitative extraction of total extracted RNA was performed by horizontal agarose gel electrophoresis and absorbance measurement at 260 and 280 nm with nanodrop machine, respectively. Total RNA sequences were used for the RT-PCR assay (Jothikumar et al., 2006; Faye et al., 2017). Multiple PVY-specific primers were used for this test (Table 1) (Boonham et al., 2002). The pair of P2 / P1 primers that amplify region 1 is not capable of separating different races. The other four primer pairs are amplified by multiplying regions of coat protein to differentiate PVY races.

Table 1- Characteristics of primers used

Name of Primer	Sequences of Primer	Type	Connection temperature	Duplicate fragment size	Position on the genome
P_1	5-TTCCAAAGTGTCCTTTGAG-3	Reverse	55	837	916-937
P_2	5-CTTCATCAAACAAACTCTTT-3	Direct			101-120
OR	5-TGTACTGATGCCACCGTCGAAC-3	Reverse	58	609	9274-9295
OF	5-TCTGGRACACATACWGTRCCR-3	Direct			8687-8710
NR	5-CCTTCATTTGAATGTGTGCCTCT-3	Reverse	58	549	9214-9236
NF	5-TCTGGAACTCAYACTGTGCCAC-3	Direct			8687-8710
OR	5-TGTACTGATGCCACCGTCGAAC-3	Reverse	58	609	9274-9295
CF	5-TCTGGAACWCATACTGTACCAA-3	Direct			8687-8708
OR	5-TGTACTGATGCCACCGTCGAAC-3	Reverse	58	609	9274-9295
NF	5-TCTGGAACTCAYACTGTGCCAC-3	Direct			8687-8710

In this study, we use Vivantis products. The cDNA was made in a final volume of 20 µL for one hour at 42 ° C in a thermocycler device. The materials used at this stage were: one microliter reverse primer (10 picomol / µl), three microliters of total RNA extraction, four microliters of MuMLV 5X buffer and one microliter of MuMLV- RT enzyme (200 units/microliter), half microliter of RNase inhibitor enzyme (40 units/microliter) and 2.5 microliters of dNTPs (10 mmol/microliter). The cDNA was amplified by PCR according to the manufacturer's instructions. The materials used for PCR were: 2.5 µl PCR 10X buffer, half Microliter MgCl2(50 molar/microliter), half microliter dNTPs(10 mmol/microliter), half microliter reverse primer(10 picomol / µl), half a microliter of enzyme Taq DNA polymerase(5 microliters/unit 5) and 2.5 microliter cDNA in a final volume of 25 microliters.

2.4. PCR-RFLP test

The P1 fragment has a cleavage site for the HincII enzyme in N and NTN strains. To this end, one microliter of the HincII enzyme and 19 microliters of enzyme buffer were poured onto the 20 microliter PCR product by P2 / P1 primers according to the manufacturer's instructions. The enzyme-containing microtubes were incubated for three hours at 37 ° C and then incubated for 3 min at 72° C.

3. Results and Discussion

In this study from 451 tomato samples collected, 13.5%, 2.6%, 20.6%, 11.3%, and 35.9% were infected with the viruses of the genus Putivirus, ArMV, PVY, CMV, Tomv, respectively, and Some were infected with two viruses (Table 2). According to the statistics presented in this study, the highest rate of viral infection was observed in the collected samples with mosaic symptoms related to PVY. The prevalence of three viruses (ToMV, PVY, and CMV) on the farm was higher than in the greenhouse. According to the observations, the activity and the number of vector aphids on the farm were much higher than in the greenhouses of Yazd. In the sampled greenhouses, planting distance was higher than that of the farm, and the plants had less contact with each other. These factors are a possible reason for the increased prevalence of this virus in tomato farms in greenhouses.

The proliferation of PVY strains in greenhouse

About 13 to 15 days after the inoculation of four selected PVY isolates on tomato plants, systemic symptoms appeared. Initial symptoms were inoculated onto the leaf, which, over time, showed signs of wrinkling and deformity throughout the plant.

Primer P1 / P2 Reaction by RT-PCR

In all four isolates tested, the P2 / P1 primer was amplified from the 837 bp fragment corresponding to the P1 protease gene (Fig. 1)

RFLP test

In the P1 fragment enzymatic digestion test in n each of the four isolates, two fragments of approximately 400 bp were generated (Fig. 2). Therefore, the result of these isolates belongs to the N or NTN group.

Table 2- Distribution of mosaic viruses in Yazd farms and greenhouses

Number of viruses infected by two viruses			The number of viruses infected only by one virus					Sampling location
PVY+CMV	ToMV+CMV	ToMV+PVY	ToMV	ArMV	PVY	CMV	Putty virus	
0	1	2	21	10	31	15	65	Greenhouse
4	3	6	40	2	62	36	97	Farm
4	4	8	61	12	93	51	162	sum

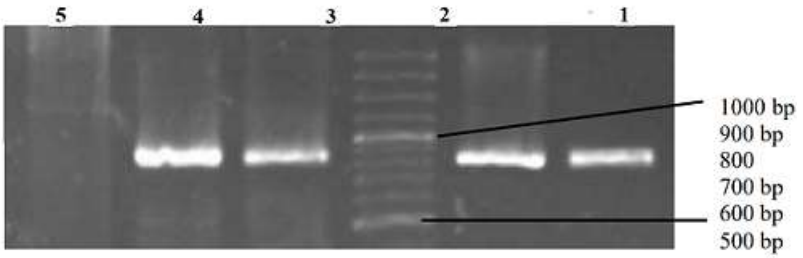

Fig. 1. Electrophoretic motifs derived from P2 / P primers: 1) T5 sample, 2) T2 sample, 3) Marker of a fermentase kilobase, 4) G1 sample 5) G2 sample and 6) Negative control sample

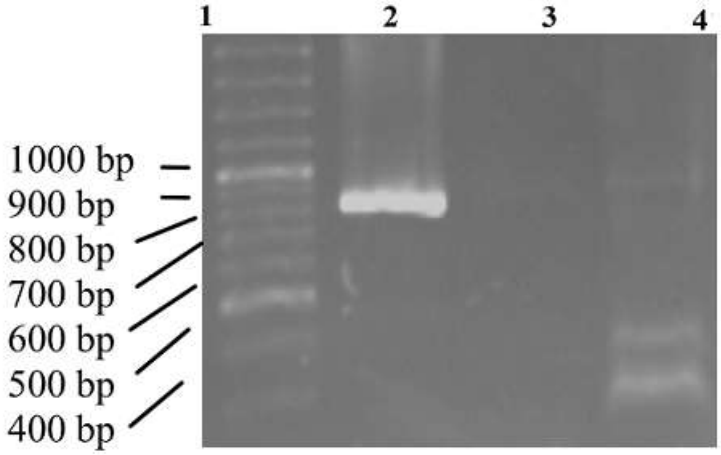

Fig. 2. Electrophoretic motifs of the product of enzyme digestion T5: 1) Marker of a fermentase kilobase (O' Gene Ruller^{TM}), 2) The PCR product contains the P1 gene, 3) Negative control, 4) PCR product digested with HincII shear enzyme

PCR-RT Reaction with Specific Primer Strains PVY

The isolates tested did not respond to specific C (CF/OR) and N (NF/NR) initiator assays. Therefore, none of the isolates belonged to race C or N. But each of the four isolates reacted with the specific primer O, and the 609 nucleotide fragments were amplified (Boonham et al., 2002). Due to the amplification of this region in all four isolates with a specific NTN primer and the creation of a 609-nucleotide fragment, this isolate belongs to the NTN strain (Fig. 3 and Fig. 4).

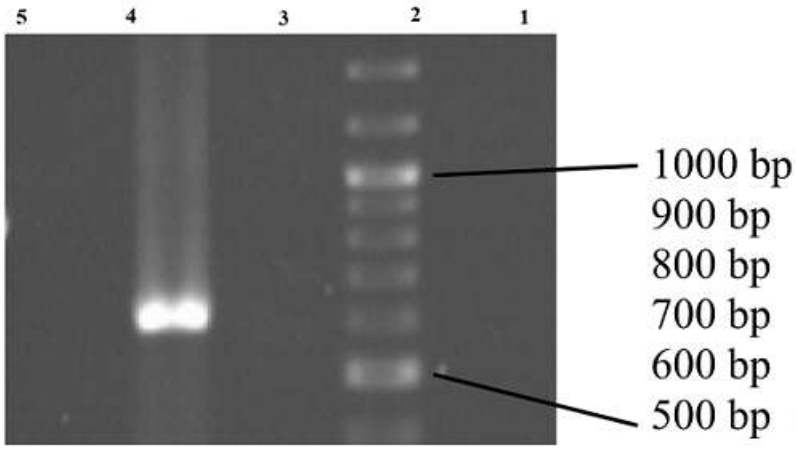

Fig. 3. Electrophoretic Motifs of T5 Isolation Reaction PCR Products with CF / OR / OF / NR / N Primers: 1) Negative control, 2) Marker of a fermentase kilobase, 3) Specific primer pair N, 4) Specific primer pair O 5) Specific primer pair C

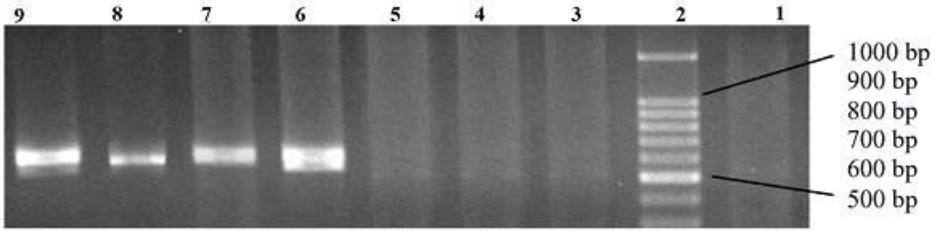

Fig. 4. Electrophoretic motifs of PCR product with specific primer pair C: 1) T5, 2) Indicator 100 pairs of game Kiaagen, 3) T2, 4) G2, 5) G1 and NTN-specific primer pair, 6) T5, 7) T2, 8) G2, 9) G1

4. Conclusion

In this study, with serological methods of tomato contamination in farms and greenhouses with CMV, PVY, ArMV, and ToMV, was evidence that PVY (20.6%) contamination was higher than the others based on the results of symptomatic studies. The results of the PCR-RT assay using specific primer pair PVY confirmed the infection with this virus. To identify the four selected isolates T5, T2, G2, and G1, the virus was generated in an RFLP test by HincII enzyme cleavage in the P1 region amplification product with a P2 / P1 primer pair of approximately 400 bp fragments. The cut in this area indicates that these isolates belong to the N or NTN race. The isolates tested did not respond to specific O and NTN (NF/OR and OF/OR) initiator assays .Therefore, considering all the results, all four isolates T5, T2, G1, and G2 belong to PVT genus NTN.

Reference

[1] Aseel, D. G., Rashad, Y. M., & Hammad, S. M. (2019). Arbuscular mycorrhizal fungi trigger transcriptional expression of flavonoid and chlorogenic acid biosynthetic pathways genes in tomato against tomato mosaic virus. *Scientific reports*, *9*(1), 1-10.

[2] Azizan, N. H., Abidin, Z. A. Z., & Phang, I. C. (2017). Study of cucumber mosaic virus gene expression in Capsicum annuum. *Sci. Herit. J*, *1*, 29-31.

[3] Boonham, N., Walsh, K., Hims, M., Preston, S., North, J., & Barker, I. (2002). Biological and sequence comparisons of Potato virus Y isolates associated with potato tuber necrotic ringspot disease. *Plant Pathology*, *51*(2), 117-126.

[4] Chung, B. Y. W., Miller, W. A., Atkins, J. F., & Firth, A. E. (2008). An overlapping essential gene in the Potyviridae. *Proceedings of the National Academy of Sciences*, *105*(15), 5897-5902.

[5] Clark, M. F., & Adams, A. N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of general virology*, *34*(3), 475-483.

[6] Faye, M., Dacheux, L., Weidmann, M., Diop, S. A., Loucoubar, C., Bourhy, H., ... & Faye, O. (2017). Development and validation of sensitive real-time RT-PCR assay for broad detection of rabies virus. *Journal of virological methods*, *243*, 120-130.

[7] Gao, F., Lin, W., Shen, J., & Liao, F. (2016). Genetic diversity and molecular evolution of arabis mosaic virus based on the CP gene sequence. *Archives of virology*, *161*(4), 1047-1051.

[8] Gulya, T. J., Shiel, P. J., Freeman, T., Jordan, R. L., Isakeit, T., & Berger, P. H. (2002). Host range and characterization of Sunflower mosaic virus. *Phytopathology*, *92*(7), 694-702.

[9] Jothikumar, N., Cromeans, T. L., Robertson, B. H., Meng, X. J., & Hill, V. R. (2006). A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *Journal of virological methods*, *131*(1), 65-71.

[10] Karimi, A. K. (1967). Potato virus diseases. *Plant Disease*, *3*, 23-32.

[11] Li, Y., Wang, Y., Hu, J., Xiao, L., Tan, G., Lan, P., ... & Li, F. (2017). The complete genome sequence, occurrence and host range of Tomato mottle mosaic virus Chinese isolate. *Virology journal*, *14*(1), 15.

[12] Ming, F., Cheng, Y., Ren, C., Suolang, S., & Zhou, H. (2019). Development of a DAS-ELISA for detection of H9N2 avian influenza virus. *Journal of virological methods*, *263*, 38-43.

[13] Otulak-Kozieł, K., Kozieł, E., & Lockhart, B. E. (2018). Plant cell wall dynamics in compatible and incompatible potato response to infection caused by Potato virus Y (PVYNTN). *International journal of molecular sciences*, *19*(3), 862.
[14] Ozaslan, M., Bas, B., Aytekin, T., & Sigirci, Z. (2006). Identification of pepper viruses by DAS-ELISA assays in Gaziantep-Turkey. *Plant Path. J*, *5*, 11-14.
[15] Shukla, D. D., Frcnkel, M. J., & Ward, C. W. (1991). Structure and function of the potyvirus genome with special reference to the coat protein coding region. *Canadian Journal of Plant Pathology*, *13*(2), 178-191.
[16] Smith, K.M. (1931a). Composite nature of certain potato viruses of the mosaic group. *Nature*, *127*(3210), 702-702.
[17] Smith K.M. (1931b). Composite nature of certain potato viruses of the mosaic group as revealed by the use of plant indicator. Proceeding of Royal Society Biological Sciences, 109:251-267.
[18] Stare, T., Ramšak, Ž., Blejec, A., Stare, K., Turnšek, N., Weckwerth, W., ... & Gruden, K. (2015). Bimodal dynamics of primary metabolism-related responses in tolerant potato-Potato virus Y interaction. *BMC genomics*, *16*(1), 716.
[19] Valli, A. A., Gallo, A., Rodamilans, B., López- Moya, J. J., & García, J. A. (2018). The HCPro from the Potyviridae family: an enviable multitasking Helper Component that every virus would like to have. *Molecular plant pathology*, *19*(3), 744-763.