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**SEROLOGICAL AND MOLECULAR DETECTION OF VIRUS INFECTIONS OF TOMATO (*Lycopersicum esculentum* Mill.) PLANTS IN AZERBAIJAN****Academician of ANAS I.M.Huseynova, S.T.Mirzoyeva,  
N.F.Sultanova, Academician of ANAS J.A.Aliyev**

The study was conducted to determine the prevalence of virus diseases in two important tomato growing regions of Azerbaijan (Goycay and Salyan) in 2014 and 2015 years. Tomato (*Lycopersicum esculentum* Mill.) plants exhibiting virus-like disease symptoms were collected in various gardens and greenhouses and tested by the lateral flow immunoassay technique (the ImmunoStrip test of Bioreba AD, Sweden and Agdia Inc., USA) and enzyme-linked immunosorbent assay (DAS-ELISA) method. As a result Tomato mosaic virus (ToMV), Tobacco mosaic virus (TMV), Cucumber mosaic virus (CMV), Tomato spotted wilt virus (TSWV), Pepper mild mottle virus (PMMoV) were detected in Tomato plants. RTR-PCR was performed with a pair of tobamovirus universal primers Tobamo 1 (5' - CGACATCAGCCGATGCAGC-3') and Tobamo 2 (5'-ACCGTTTTCGAACCGAGACT-3') using the total RNA extracted from the symptomatic leaves. The PCR analysis resulted in successful amplification of expected size (~320 bp) amplicons from the infected samples which are characteristic for tobamovirus.

**Keywords:** *Lycopersicum esculentum* Mill., virus diseases, immunostrips, immunofluorescent analysis (IFA), enzyme-linked immunosorbent assay (ELISA), RT-PCR

**Introduction**

Tomato (*Solanum lycopersicum* L.) is the most widely cultivated vegetable all over the world. In Azerbaijan tomato is a very popular and an important vegetable crop which is consumed on nearly daily basis in every household. However, its production is directly connected with an elevated occurrence of pathogens limiting the production efficiency of the vegetable [3, 9, 12]. One of the most important biotic constraints affecting tomato production is virus infection.

Currently more than 50 virus species infecting tomato plants are known [1]. In virosis plant growth almost stops, fruit and roots become damaged, pigment stains are observed in various organs (especially in leaves and fruit), metabolism (respiration, photosynthesis, ion exchange etc.) is destroyed and eventually the plant perishes [6]. Tomato mosaic virus (ToMV), Tobacco mosaic virus (TMV), Cucumber mosaic virus (CMV), Tomato spotted wilt virus (TSWV) and Pepper mild mottle virus (PMMoV) are examples of viroses caused by viruses affected tomato plants [4]. Stains in blades, yellowing and

dark greenish blade areas emerge in tomato plants infected with tobamoviruses [10]. The shape of leaves become like in fern plants [2]. Bleaching occurs in the flowers of infected plants [7]. Infection can spread by seeds of a diseased plant [11]. So only a few diseased seedlings are enough to destroy the whole field, because to treat the plant that has already infected with the pathogen is impossible [5]. As preventive measures it is important to monitor plants in fields and greenhouses and conduct pathogen test in seeds intended for sowing [8].

The presented work is devoted to detection of virus diseases affected tomato plants in Azerbaijan using modern serological and molecular techniques.

**Materials and methods**

**Collecting plant material and serological diagnostics of virus diseases.** Phytopathological monitorings were carried out and plant samples with characteristic symptoms were collected from various gardens and greenhouses of Shekek, Mirti, Karakhidir, Hajikend, Karabaggal, and

Lekchilpag villages of Goychay district and Yenikend and Garabagli villages of Salyan district during autumn and spring months in of 2014 and 2015 years. Viral diseases were expressed by symptoms of leaf deformation and discoloration, leaf distortion, bubbly, yellowing, mottling and different mosaic patterns, young leaves may curl downwards, brown streaked, leaves shortening the life-span, extensive leaf coloring and stunting, leaf size reduction, may cause a striking yellow mosaic, whereas other strains may cause leaf malformation and "fernleafing," (leaf rolling), formation of necrotic lesions, light- and dark-green mosaic pattern on leaves, yellow ringspots and vein necrosis of leaves, foliar necrosis and brown streaking of the stems and petioles, fruit are severely mottled, making them unmarketable, destroyed and reduced fruit quality and growth reduction were observed.

The collected tomato samples were tested on ToMV, TMV, CMV, TSWV, PMMoV viruses with a modern serological method and diagnostic test-system using specific test-bands and immunoenzyme analysis (Bioreba AG, Swedish v  Agdia Inc., USA) allowing the simultaneous analysis of a large number of samples. For this purpose, extracts from leaf samples were prepared using adequate buffers according to the protocol. Specific agristrips were tested on each virus and analysis was performed using specific antibodies (IgG) placed before hand on the tablet prepared for ELISA. The enzymatic reaction occurring on the tablet was first assessed visually by color change. The virus concentration was determined spectrophotometrically (Stat Fax Microplate, Awareness Technology, USA) with positive samples.

**Total RNA extraction and reverse transcription.** Total RNA was extracted from 30-50 mg of fresh leaf tissue using the solution TriReagent (Life Technologies) according to the manufacturer's recommendation to isolate total RNA from plants. Leaf samples were homogenized and a mixture of TriReagent and chlorophorm was used to extract RNA. Isopropanol was used to precipitate RNA from the aqueous layer. The obtained pellet was resuspended in RNase-free water. Subsequently, 5 µg of the RNA was loaded to denatured agarose gel to

estimate the integrity of the RNA. Purity and concentration of the extracted RNA samples were tested spectrophotometrically. Then the RNA samples were analysed with RT-PCR at the same time were subjected to RT-PCR analysis. RT-PCR was performed in the reaction mix containing 4 µl RNA, 1.5 µl Tobamovirus universal primer (TobRT do2), 1.5 µl d NTP (25 mM), 4 µl RT (5x) buffer, 1 µl M-MLV (RT enzyme), 8 µl ddH<sub>2</sub>O. The reaction was carried out for 1 h, at 42°C. To stop the reaction the samples were kept at 65°C for 10 min.

**Amplification by PCR.** After the electrophoretic analysis RT-PCR products were tested using PCR method. The mixture containing 2.5µl Tampon (10x), 3 µl MgCl (50mM), 0.5 µl Tobamovirus universal primer (Tob RT up 1 and TobRT do2), 3 µl dNTP (2.5 mM), 13.5 µl dd H<sub>2</sub>O, 0.3 µl Tag polymerase (for one sample) was prepared for the reaction. The volume of the total reaction mixture for one sample was 25 µl (5µl RNA sample + 20 µl reaction mixture). The samples were placed in PCR apparatus (DNA Thermal amplificator), programmed according to the protocol. and then PCR was performed (First DNA chain denaturation was performed at 95°C, 35 cycle elongation at 94°C for 1 min, at 65°C for 45 sec, at 70°C for 1 min and synthesis was completed at 72°C for 10 min). PCR conditions were 2 min at 94°C as an initial denaturation step, followed by 35 cycles of 1 min at 94°C, 45 sec at 65°C and 1 min at 72 °C, and an additional elongation step of 10 min at 72°C. The amplicons were run in a 3% agarose gel with ethidium bromide and recorded digitally using UV-Gel Doc system (UK).

## Results and discussion

The large crop survey to evaluate the incidence of virus diseases of tomato were realized during autumn and spring months in of 2014 and 2015 years. Surveys were conducted in different villages of Goychay and Salyan districts. During surveys virus-like symptomatic samples plants reminiscent virus infections were collected. Symptomless plants were also sampled as negative control.

The collected tomato samples were analysed using various indicator-serological methods. Re-

sults showed that *Tomato mosaic virus (ToMV)*, *Tobacco mosaic virus (TMV)*, *Cucumber mosaic virus (CMV)*, *Tomato spotted wilt virus (TSWV)* and *Pepper mild mottle virus (PMMoV)* widely spread in tomato plants from Goychay and Salyan. Immunostrip-test bands and the results

of IFA DAS-ELISA completely coincided (fig.1).

Collected tomato samples from Salyan and Goychay were analyzed using specific agristrips (test strips) and specific ELISA kits for each virus (table 1).



**Fig. 1.** Detection of *ToMV*, *TMV*, *CMV*, *TSWV* viruses in Tomato (*Solanum lycopersicum* Mill.) plants using specific agristrips (on-site test) and Enzyme-linked immunosorbent assay (DAS-ELISA)-test system

*Table 1*

**Results of the Agristrips and ELISA analysis of collected tomato samples**

| №  | Name of plant samples | Varieties | Simpling sites | Results              |   |     |   |      |   |       |   |     |   |
|----|-----------------------|-----------|----------------|----------------------|---|-----|---|------|---|-------|---|-----|---|
|    |                       |           |                | Agristrips/DAS-ELISA |   |     |   |      |   |       |   |     |   |
|    |                       |           |                | ToMV                 |   | TMV |   | TSWV |   | PMMoV |   | CMV |   |
| 1  | Tomat 1.1             | Pandaroza | Salyan         | -                    | - | +   | + | -    | - | +     | + | +   | + |
| 2  | Tomat 1.2             | Pandaroza | Salyan         | -                    | - | +   | + | -    | - | +     | + | +   | + |
| 3  | Tomat 1.3             | Pandaroza | Salyan         | -                    | - | +   | + | +    | + | +     | + | -   | - |
| 4  | Tomat 1.4             | Pandaroza | Salyan         | -                    | - | +   | + | +    | + | +     | + | -   | - |
| 5  | Tomat 2.1             | Berberena | Salyan         | -                    | - | +   | + | -    | - | +     | + | +   | + |
| 6  | Tomat 2.2             | Berberena | Salyan         | -                    | - | -   | - | -    | - | +     | + | +   | + |
| 7  | Tomat 2.3             | Berberena | Salyan         | -                    | - | +   | + | +    | + | +     | + | +   | + |
| 8  | Tomat 2.4             | Berberena | Salyan         | -                    | - | -   | - | -    | - | +     | + | +   | + |
| 9  | Tomat 2.5             | Berberena | Salyan         | -                    | - | +   | + | -    | - | -     | - | +   | + |
| 10 | Tomat 3.1             | Arletta   | Salyan         | -                    | - | +   | + | +    | + | +     | + | -   | - |
| 11 | Tomat 3.2             | Arletta   | Salyan         | -                    | - | +   | + | -    | - | +     | + | -   | - |
| 12 | Tomat 3.3             | Arletta   | Salyan         | -                    | - | +   | + | +    | + | +     | + | +   | + |
| 13 | Tomat 1.1             | Berberena | Goychay        | -                    | - | -   | - | -    | - | -     | - | +   | + |
| 14 | Tomat 1.2             | Berberena | Goychay        | -                    | - | -   | - | -    | - | -     | + | -   | - |
| 15 | Tomat 2               | Berberena | Goychay        | -                    | - | -   | - | -    | - | -     | - | -   | - |
| 16 | Tomat 3               | Berberena | Goychay        | -                    | - | -   | - | -    | - | -     | - | -   | - |
| 17 | Tomat 4.1             | Berberena | Goychay        | -                    | - | -   | - | -    | - | -     | - | -   | - |
| 18 | Tomat 4.2             | Berberena | Goychay        | -                    | - | -   | - | -    | - | -     | - | -   | - |
| 19 | Tomat 5.1             | Berberena | Goychay        | -                    | - | -   | - | -    | - | -     | - | -   | - |
| 20 | Tomat 5.2             | Berberena | Goychay        | -                    | - | -   | - | -    | - | +     | - | -   | - |
| 21 | Tomat 6.1             | Melodiya  | Goychay        | +                    | + | -   | - | -    | - | +     | - | -   | - |
| 22 | Tomat 6.2             | Melodiya  | Goychay        | -                    | - | -   | - | -    | - | -     | - | -   | - |

*Tobacco mosaic virus* related to tospoviruses and *Tomato spotted wilt virus* related to Tospoviruses were detected as a mixed infection (TMV + TSWV) in 5 Tomato (*Solanum lycopersicum* L.) samples. *Tomato spotted wilt virus* related to Tospoviruses and *Cucumber mosaic virus* related to cucumoviruses as a mixed infection (TMV + CMV) were found in 6 samples. In 1 sample a mixed infection of three viruses (TMV+ TSWV + CMV) and in 2 samples a mixed infection of four viruses (TMV+ TSWV + CMV + PMMoV) were observed.

The degree of natural distribution (in %) of the virus infection detected in tomato plants grown in Salyan district established by phytopathological monitoring has been presented in fig. 2. From 12 samples showing positive results i.e. infected plants *Tobacco mosaic virus* (TMV) were detected in 10 (83%), *Tomato spotted wilt virus* (TSWV) in 5 (42%), *Pepper mild mottle virus* (PMMoV) in 11 (95%) and *Cucumber mosaic virus* (CMV) in 8 (67%) samples.

To identify virus diseases detected in tomato plants, RNAs were isolated from positive samples of the serological analysis and tested with RT-PCR method. Tobamo 1 (5'-CGACATCAGCCGATGCAGC-3' and Tobamo 2 (5'-ACCGTTTTTCGAACCGAGACT-3') universal primers for tobamoviruses were chosen for RT-PCR amplification. Results of the identification of viruses detected in tomato by the RT-PCR technique are presented in fig. 2.

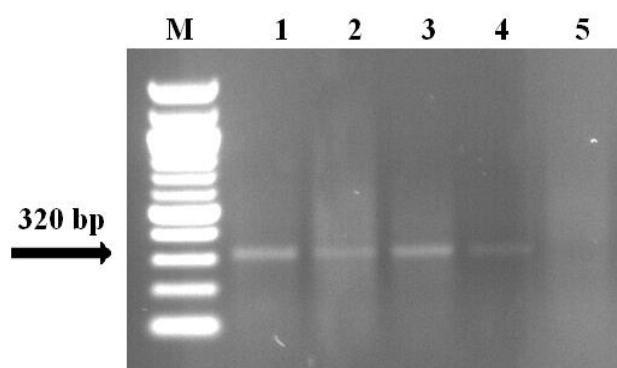


**Fig. 2.** RT-PCR analysis of infected tomato plants collected during phytopotological monitoring carried out in Salyan district. M-100 bp DNA ladder, 1-pandaroza #1.1; 2-pandaroza #1.2; 3-pandaroza #1.3; 4-pandaroza #1.4; 5-healthy plant (negative control)

Symptomless plants gave no amplification while expected 320 bp amplicons were obtained from the infected tomato samples which are characteristic for tobamovirus.

Thus, the obtained results confirm that globalization of the agricultural system and international exchange of sowing materials create conditions for the introduction of viral infections to new regions. Periodically performing phytopathological monitorings to detect the viruses, choosing healthy plants, certification of sowing materials could particularly prevent virus diseases. To control diseases caused by viruses initial diagnostics of these pathogens, taxonomic characterization and identification of host plant spectrum and insect vector are needed necessary.

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## REFERENCES

1. Blancard D. (2012) Tomato diseases identification, biology and control second edition, 561-627.
2. Glala A.A., Hoda A.M., Fawzi Z.F. (2005) Improving tomato plant growth, health, earliness, productivity and fruit quality by chemically induced systematic resistance. *Journal of Applied Sciences Research*. **1**: 362-372.
3. Horna D., Smale M., Falck-Zepeda J. (2006) Assessing the potential economic impact of genetically modified crops in Ghana: Tomato, Garden Egg, Cabbage & Cassava. *PBS report*, Oct., 2006.
4. Janjić N., Živojin E. (2012) Morphometric and Anatomical-Histometric Characteristics of two varieties of the species *Solanum lycopersicum* L. infected by *Cucumber Mosaic Virus*. *Agroznanje*, **13(4)**:591-602.
5. Joseph N., Nigel J.T., Jitender Y., Haytham A., James L., Terry A., Graham T., Claude M.F. (2005) Application of FTA technology for sampling, recovery and molecular characterization of viral pathogens and virus-derived transgenes from plant tissues. *Virology Journal*, **2**:45.
6. Hristova K.D., Yanev D.T. (2010) Spectral reflectance technique for detection of viral infections on tomato plants (*Lycopersicon esculentum* L.). *Six Scientific Con-*

ference with International Participation Space, Ecology, Safety, Sofia, Bulgaria.

7. Madhusudhan K.N., Nalini M.S., Prakash H.S., Shetty H.S. (2005) Effect of inducers against Tobamovirus infection in tomato and bell pepper. *International Journal of Botany*, **1**: 159-61.

8. Mnari-Hattab M., Ezzaier K. (2006) Biological, serological, and molecular characterization of Pepper mild mottle virus (PMMoV) in Tunisia. *Tunisian Journal of Plant Protection*, **1**: 1-12.

9. Osei M. K., Akromah R., Shih S. L., Green S. K. (2010) Evaluation of some tomato germplasm for resistance to Tomato yellow Leaf curl Virus disease (TYLCV) in Ghana. *Aspects of Applied Biology*, **96**: 315-323

10. Spence N.J., Scaly I., Mills P.R., Foster G.D. (2001) Characterization of a Tobamovirus from railing Petunias. *Eur. J. Plant Pathol.*, **107**: 633-638.

11. Sunpapao A. (2013) Virus-Induced Symptoms in Plants: A review of interactions between viral trafficking and RNA silencing. *Phillip Agric Scientist*, **96(2)**: 210-218.

12. Wolff H. (1999) Economics of tomato production with special reference to aspects of plant protection: a case study of two tomato production systems in Brong-Ahafo region, Ghana prepared for: Ghanaian-German Project for Integrated Crop Protection, GTZ, Eschborn. 131 p.

*Institute of Molecular Biology and Biotechnology  
of ANAS*

*huseynova-i@botany-az.org*

## AZƏRBAYCANDA TOMAT (*LYCOPERSICUM ESCULENTUM* MILL.) BİTKİSİNİN VİRUS XƏSTƏLİKLƏRİNİN SEROLOJİ VƏ MOLEKULAR METODLARLA TƏYİNİ

**İ.M.Hüseynova, S.T.Mirzəyeva, N.F.Sultanova, C.Ə.Əliyev**

Azərbaycanda virus xəstəliklərinin yayılmasını aşkar etmək məqsədilə 2014-2015-ci illərdə tomat bitkisinin geniş becərildiyi Göyçay və Salyan rayonlarında tədqiqatlar aparılmışdır. Virus xəstəliyinin xarakterik əlamətlərinə malik tomat nümunələri (*Lycopersicum esculentum* Mill.) müxtəlif əkin sahələrindən və istixanalardan toplanaraq spesifik immunostriplər (Bioreba AD, İsveç və Agdia Inc., ABŞ) və immunoferment analiz (DAS-ELISA) metodları ilə yoxlanılmışdır. Nəticədə tomat bitkisinə *Tomato mosaic virus* (ToMV), *Tobacco mosaic virus* (TMV), *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV), *Pepper mild mottle virus* (PMMoV) aşkar edilmişdir. Xəstə yarpaq nümunələrindən ayrılmış RNT ekstraktları *Tobamo 1* (5'-CGACATCAGCCGATGCAGC-3') və *Tobamo 2* (5'-ACCGTTTTCGAACCGAGACT-3') universal praymerlərdən istifadə etməklə RT-PZR metodu ilə yoxlanılmışdır. PZR nəticəsində ~320 bp ölçülü fraqmentlər sintez edilmişdir ki, bu da həmin nümunələrin tobamoviruslarla yoxlandığını göstərir.

**Açar sözlər:** *Lycopersicum esculentum* Mill., virus xəstəlikləri, immunostriplər, immunoferment analiz (İFA), RT-PZR

## ДЕТЕКЦИЯ ИНФЕКЦИОННЫХ ЗАБОЛЕВАНИЙ РАСТЕНИЙ ТОМАТА (*LYCOPERSICUM ESCULENTUM* MILL.) В АЗЕРБАЙДЖАНЕ СЕРОЛОГИЧЕСКИМИ И МОЛЕКУЛЯРНЫМИ МЕТОДАМИ

**И.М.Гусейнова, С.Т.Мирзоева, Н.Ф.Султанова, Дж.А.Алиев**

Исследования были проведены в 2014 и 2015 годах с целью определения распространенности вирусных заболеваний в двух основных районах выращивания томатов в Азербайджане (Гейчай и Сальян). Образцы растений томата (*Lycopersicum esculentum* Mill.) с характерными симптомами вирусного заболевания, собранные из различных оранжерей и теплиц, были исследованы специфическими иммунострипами (Bioreba AD, Швеция и Agdia Inc., США) и иммуноферментным анализом (DAS-ELISA). В результате у томата были выявлены *Tomato mosaic virus* (ToMV), *Tobacco mosaic virus* (TMV), *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV) и *Pepper mild mottle virus* (PMMoV). ОТ-ПЦР проводили с помощью одной пары универсальных праймеров для tobamovirusов *Tobamo 1* (5'-CGACATCAGCCGATGCAGC-3') и *Tobamo 2* (5'-ACCGTTTTCGAACCGAGACT-3') с использованием тотальной РНК, выделенной из симптоматических листьев. В результате успешной амплификации методом ПЦР из зараженных образцов были получены ампликоны размером ~ 320 п.н., что характерно для tobamovirusов.

**Ключевые слова:** *Lycopersicum esculentum* Mill., вирусные заболевания, иммунострипы, иммуноферментный анализ (ИФА), ОТ-ПЦР