Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2024, Vol. 8 (1), 31-37
https://doi.org/10.29329/ijiaar.2024.656.3

Original article

Serological Analyses of Viruses Presence on Tomato Collection from the Gene Bank of the Republic of Srpska

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Abstract

Testing for virus presence on tomato (*Solanum lycopersicum* L.) collection from the Gene Bank of the Republic of Srpska was conducted during the growing season of 2023 in the greenhouse of the Institute of Genetic Resources, University of Banja Luka, Bosnia and Herzegovina. Thirty samples were taken and preliminary tested for presence of 3 viruses: TSWV (Tomato spotted wilt virus, Tospovirus), TBRV (Tomato black ring virus, Nepovirus), ToBRFV (Tomato brown rugose fruit virus, Tobamoviruses) with ELISA (Bioreba) test. Fourteen samples were positive for TSWV presence and negative for other two viruses. The previous research has been conducted on the presence of TSWV on conventional tomato varieties in the open field and in the greenhouse, but never on the tomato accessions from the Gene Bank that represent domesticated tomato germplasm.

Keywords: TSWV, TBRV, ToBRFV, tomato, Gene Bank.

Received: 04 September 2023 * **Accepted:** 15 March 2024 * **DOI:** https://doi.org/10.29329/ijiaar.2024.656.3

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INTRODUCTION

Tomato spotted wilt virus (TSWV) is one of the most widespread plant viruses and also have the largest host-range. The current list of TSWV hosts consists of 1090 plants species (Parrella, et al. 2003) both monocotyledonous and dicotyledonous plants (Moyer, 1999) and weed species. Tomato spotted wilt virus (TSWV) belongs to the genus Tospovirus of the family Bunyaviridae. TSWV is transmitted by thrips in a circulative and propagative manner (Pappu, 2008). This virus is one of the most destructive viruses, responsible for numerous epidemics in different regions of the world, and cause heavy economic losses (Parrella et al., 2003). In the Republic of Srpska (district of Bosnia and Herzegovina), until now TSWV was detected in pepper plants from greenhouses and tobacco plants from open field (Delić et al., 2017) and also confirmed the presence of western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) and tobacco thrips, *Thrips tabaci* (Linderman) in greenhouses in Herzegovina region (Trkulja et al., 2013; Kohnić et al., 2006). The highly polyphagous nature, the efficiency of virus transmission and the biological activity of its vectors, the rapidity with which new variants arise, and difficulties in the control of the vectors, make TSWV one of the most feared plant viruses by growers of agricultural crops. Preventive and integrated cultural practices such as the eradication of weed hosts able to serve as virus reservoirs, combined with vector management strategies, play a crucial role in the control of the virus.

Tomato black ring virus (TBRV) belong to the Nepoviruses group (nematode-transmitted virus) that infect many plant families: annual, perennial and woody plants, economically important crop species like: grapevine, cherry, apricot, peach, berry-fruits, different ornamental plants and weeds, and solanaceous species like: potato, pepper, tobacco and tomato (Edwardson et al., 1997). They cause of economic importance diseases in a wide range of cultivated, and concerned as quarantine worldwide (Šneideris et al., 2012). Their wide host range combined with ability to be transmitted by nematodes, seed and/or pollen makes them severe problem, hard to eradicate and control (Murant, 1981; Card et al., 2007). TBRV is transmitted both through seeds and by free-living nematodes *Longidorus elongatus* and *Longidorus attenuatus* (Harrison et al., 1961; Brown et al., 1989) by feeding on roots. The virus has been reported in Europe, North and South America, India and Japan (Harper et al., 2011), Australia and New Zealand (Šneideris et al., 2012). Known until now, Tomato black ring virus (TBRV) was previously detected on potato and grapevine in some parts of Yugoslavia. Isolates from sugar beet, pepper and tobacco were found in North Bosnia, the second tobacco isolate in Herzegovina, and the potato isolate in West Bosnia (Buturović et al., 1979).

Tomato brown rugose fruit virus (ToBRFV) belong to the genus *Tobamovirus*, and has been identified from tomato plants (Luria et al., 2017; Salem et al., 2016). ToBRFV was discovered in greenhouse tomato plants grown in Jordan and its first outbreak was in Israel (Salem et al., 2016). To date, the virus has been reported in at least 35 countries across four continents in the world. ToBRFV

infects tomato as the primary host and considered the most serious threat to tomato production in the world. Recently, virus has caused devastating disease outbreaks in tomato production areas in many countries, resulting in a severe reduction in yield (Avni et al., 2021; EPPO, 2020; Jones, 2021; Oladokun et al., 2019). ToBRV is transmitted by mechanical contact, propagation material, plant debris, contaminated soil, growing media, circular water, workers farming activities and tools (Oladokun et al., 2019). Italy and Slovenia are the nearest counties to Bosnia and Herzegovina that have identified the presence of this virus (Panno et al., 2019). EPPO Working Party on Phytosanitary Regulations and Council agreed that ToBRFV should be added to the A2 List of pests recommended for regulation as quarantine pests in 2020.

MATERIALS and METHODS

Plant material

The research was conducted during the growing season of 2023 on 30 tomato accessions from the Gene Bank of the Republic of Srpska in Bosnia and Herzegovina. Containerized tomato seedlings were produced according to standard agricultural technology in the unheated glass greenhouse at the Faculty of Agriculture, University of Banja Luka. Total of 60 plants (2 plants per accession) were planted in pots in a tunnel-type polypropylene greenhouse at Institute of Genetic Resources, Umiversity of Banja Luka (158 m altitude, 44.774971 latitude and 17.211463 longitude) with total area of 115 m^2. Fertilization was applied before planting and during vegetation. Plants were maintained using standard horticultural practices such as trellising and pinching. Insecticide was sprayed twice on the plants after transplanting in greenhouse to exterminate any thrips vector.

During the growing season of 2023 monitoring and sample collection were carried out in the greenhouse of the Institute of Genetic Resources, University of Banja Luka. Leaf samples were collected on 105^{th} day of vegetation when fruits on the 1^{st} truss were ripe. All samples were collected in duplicate for technical replications. One sample for DAS-ELISA testing included leaves from the both plants of the same accession. All plants were tested for the presence of all 3 viruses, no matter if leaf symptoms were present or not.

Sample preparation

Fresh leaf samples were homogenized using Bioreba extraction bags. Prepared samples were stored at temperature 4°C overnight.

Serological analysis

Prepared samples were analyzed by double-antibody sandwich enzyme-linked immunsorbent assay (DAS-ELISA) using commercial diagnostic kits (Bioreba AG, Reinach, Switzerland) against: TSWV (Tomato spotted wilt virus, Tospovirus), TBRV (Tomato black ring virus, Nepovirus) and

ToBRFV (Tomato brown rugose fruit virus, Tobamoviruses) according to manufacturer's instructions. Commercial positive and negative controls (Bioreba) were included in each assay. ELISA reactions were read for absorbance at 405 nm using a HiPo MPP-96 Microplate Reader (BioSan, Lithuania). Also, yellow color development was assessed visually after 30 and 60 minutes.

Statistical analysis

All obtained results were analyzed by standard descriptive statistical methods. Samples with absorbance values twice higher than in healthy uninfected negative controls were considered positive for virus infection.

RESULTS and DISCUSSION

The main aim of this research was to investigate the presence of 3 commercialy important viruses in tomato accessions that have been multiplicated for seed collection in the Gene Bank. Leaf samples were collected from each accession, both from symptomatic and asymptomatic plants. Mild symptoms like leaf chlorosis and leaf nerve yellowing were visible during sample collection and other symptoms were not noticed.

A total of 30 accessions of local tomato germplasm were analysed for viruse presence using DAS-ELISA test. The Gene bank code, local name, fruit colour, location of origin and the presence of TSWV for all 30 accessions are shown in Table 1.

Table 1. Gene bank code, local name, fruit colour, location of origin and TSWV presence for 30 tomato accessions

No.	Gene bank code	Local name	Fruit colour	Location of origin	TSWV presence
1.	GB00415	'Paradajz'	red	unknown	-
2.	GB00498	'Paradajz'	red	unknown	-
3.	GB00545	'Amerikanac'	red	Karanovac, Petrovo municipality	-
4.	GB00548	'Amerikanac'	red	Dragočaj, Banja Luka municipality	-
5.	GB00874	'Amerikanac'	red	Karanovac, Petrovo municipality	-
6.	GB00875	'Jabučar'	red	Karanovac, Petrovo municipality	+
7.	GB01092	'Žuti'	yellow	Kostajnica municipality	-
8.	GB01106	'Volujsko srce'	pink	Dragočaj, Banja Luka municipality	+
9.	GB01107	'Paradajz'	red	Dragočaj, Banja Luka municipality	+
10.	GB01108	'Paradajz'	red	Karanovac, Petrovo municipality	+
11.	GB01109	'Sitni'	red	Konopljišta, Petrovo municipality	+
12.	GB01110	'Sitni'	red	Karanovac, Petrovo municipality	-
13.	GB01122	'Domaći trebinjski'	red	Zasad (Obalina), Trebinje municipality	+
14.	GB01123	'Paradajz'	red	Vrela, Petrovo municipality	-
15.	GB01124	'Jabučar'	red	Gornji lokvanj, Zvornik municipality	+
16.	GB01125	'Amerikanac'	red	Čađavica, Novi Grad municipality	+
17.	GB01126	'Trebinjski sitni'	red	Gornje Police, Trebinje municipality	+
18.	GB01128	'Jabučar'	red	Slatina, Laktaši municipality	+
19.	GB01129	'Žuti'	yellow	Slatina, Laktaši municipality	-
20.	GB01132	'Žuti amerikanac'	yellow	Konopljišta, Petrovo municipality	-
21.	GB01238	'Žuti'	yellow	unknown	-
22.	GB01239	'Volovsko srce'	pink	Kosjerovo, Laktaši municipality	-
23.	GB01240	'Trebinjski jabučar'	red	Donji Rakani, Novi Grad municipality	+
24.	GB01323	'Paradajz'	red	unknown	+
25.	GB01324	'Paradajz'	red	unknown	-
26.	GB01325	'Paradajz'	red	unknown	-
27.	GB01345	'Sitni'	red	Jelićka, Prijedor municipality	-
28.	GB01353	'Amish paste'	red	unknown	+
29.	GB01414	'Paradajz'	red	unknown	-
30.	GB01421	'Paradajz'	red	unknown	+

DAS-ELISA positive tests resulted in 46.67% (14/30) of TSWV infected plants, most of them being asymptomatic plants. These results showed a high infection on TSWV, which presence is already detected in Bosnia and Herzegovina. Also, the presence of *F. occidentalis*, vector of TSWV, enhances possibilities for rapid dissemination of this virus in greenhouse. The presence of TSWV infected plants was not dependant on the location of origin of accession, thereby we can conclude that the TSWV was widespread all over the investigated region of origin.

Other two viruses, TBRV and ToBRFV were negative in DAS-ELISA test for selected 30 tomato accession. All nepoviruses are transmitted through soil by free-living nematodes of *Longidorus* and *Xiphinema* species, feeding on roots. Considering that the soil substrate Klasman TS3 (Germany) was used for plant production in the greenhouse, and TBRV tends to be transmitted by seeds and pollen and has been reported in some counties in Europe in the RNQP (Regulated non-quarantine pest) status, the goal was to check whether the virus is present in the seed collection from the Gene Bank of the Republic of Srpska. Testing the presence of ToBRFV was done since there were reported infections in the neighboring counties, it is easily transmitted by seeds and other propagation material and it is on the A2 List of pests recommended for regulation as quarantine pests.

This paper represents preliminary work and first results of virus presence in domesticated tomato germplasm in the Gene Bank of the Republic of Srpska, Bosnia and Herzegovina.

CONCLUSION

During multiplication for seeds collection, regular procedure includes testing for the quality and health status before long-term storage in the Gene Bank of the Republic of Srpska and later utilization of those accessions for research and breeding activities. Analized tomato collection tested positive for TSWV (14/30) but negative for TBRV and ToBRFV presence. However, this pilot study represents background for a wider survey of TSWV, TBRV and ToBRFV presence and thrips species as potential insect-vectors in Bosnia and Herzegovina.

REFERENCES

Avni, B., Gelbart, D., Sufrin-Ringwald, T., Zinger, A., Chen, L., Machbash, Z. (2021). Tomato genetic resistance to tomato viruses in compromised. *Acta Horticulturae*, 1316, 89-98.

Buturović, D., Grbelja, J., Jelić, Ž. (1979). On the geographical distribution of tomato black ring virus in Yugoslavia. *Acta Botanica Croatica*, 38(1), 9-12.

Brown, D. F., Murant, A. F., Trudgill, D. L. (1989). Differences between isolates of the English serotype of tomato black ring virus in their transmissibility by an English population of *Longidorus attenuatus* (Nematoda: Dorylaimoidea). *Revue Nematologie*, 12, 51–56.

Card, S. D., Pearson, M. N., Clover, G. R. G. (2007). Plant pathogens transmitted by pollen. *Australasian Plant Pathology*, 36, 455–461.

Delić, D., Balech, B., Radulović, M., Đurić, Z., Lolić, B., Santamaria, M., Đurić, G. (2017). Molecular identification of Tomato spotted wilt virus on pepper and tobacco in Republic of Srpska (Bosnia and Herzegovina). *European Journal of Plant Pathology*, 134(3).

Edwardson, J. R., Christie, R. G. (1997). Viruses infecting peppers and other Solanaceous crops. Gainesville, USA, 337–390.

EPPO (2020). Tomato bron rugose fruit virus, EPPO Bulletin, 50, 529-534.

Harper, S. J., Delmiglio, C., Ward, L. I., Clover, G. R. G. (2011). Detection of Tomato black ring virus by realtime one-step RT-PCR. *Journal of Virological Methods*, 171(1), 190–194.

Harrison, B. D., Mowat, P., Taylor, C. E. (1961). Transmission of a strain of tomato black ring virus by *Longidorus elongatus* (Nematoda). *Virology*, 4(4), 480–485.

Kohnić, A., Ostojić, I., Karić, N. (2006). Vegetable pests in greenhouses in territory of Herzegovina. *Radovi Poljoprivrednog Fakulteta Univerziteta u Sarajevu*, 51(2), 139–140.

Luria, N., Smith, E., Reingold, V., Bekelman, I., Lapidot, M., Levin, I., Elad, N., Tam, Y., Sela, N., Abu-Ras, A., Ezra, N., Haberman, A., Yitzhak, L., Lachman, O., Dombrovsky A. (2017). A new Israeli Tobamovirus isolate infects tomato plants harboring tm-22 resistance genes. *PLoS One*, 12(1).

Jones, R.A.C. (2021). Global plant viruses disease pandemic and epidemic. *Plants*, 10, 233.

Moyer, J.W. (1999). *Encyclopedia of virology, Second edition*, 1803-1807.

Murant, A. F. (1981). *Nepoviruses, Handbook of plant virus infections/Comparative Diagnosis*. Amsterdam, Netherlands, 197–238.

Oladokun, J. O., Halabi, M. H., Barua, P., Nath, P. D. (2019). Tomato brown rugose fruit disease: current distribution. Knowledge and future prospects, *Plant Pathology*, 8, 1579-1586.

Panno, S., Caruso, A. G., Davino, S. (2019). First report of tomato brown rugose fruit virus on tomato crops in Italy. *Plant Disease*, 103, 1443.

Pappu, H. R. (2008). *Tomato Spotted Wilt Virus, Encyclopedia of Virology (Third Edition)*, 133-138.

Parrella, G., Gognalons, P., Gebre-Selassiè, K., Vovlas, C., Marchoux, G. (2003). An update of the host range of Tomato Spotted Wilt Virus. *Journal of Plant Pathology*, 85(4), 227-264.

Salem, N., Mansour, A., Ciuffo, M., Falk, B. W, Turina, M. (2016). A new tobamo virus infecting tomato crops in Jordan. *Archivesof Virologz*, 161, 503-506.

Trkulja, V., Mihić Salapura, J., Ćurković, B., Stanković, I., Bulajić, A., Vučurović, A., Krstić, B. (2013). First report of tomato spotted wilt virus on gloxinia in Bosnia and Herzegovina. *Plant Disease*, 97, 429.

Šneideris, D., Zitikaitė, I., Žižytė, M., Grigaliūnaitė, B., Staniulis, J. (2012). Identification of nepoviruses in tomato (*Lycopersicon esculentum* Mill.). *Agriculture*, 99(2), 173–178.