Original article

Identification of *Tomato black ring virus* from tomato plants grown in greenhouses in Saudi Arabia

Anas Mohammed Al-Shudifat *⇑, Ibrahim Mohammed Al-Shahwan, Mohammad Ali Al-Saleh, Omer Ahmed Abdalla, Mahmoud Ahmed Amer

Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2450, Riyadh, 11451, Saudi Arabia

**Article info**

**Article history:**
Received 9 November 2020  
Revised 13 January 2021  
Accepted 14 January 2021  
Available online 28 January 2021

**Keywords:**  
DAS-ELISA  
RT-PCR  
Saudi Arabia  
TBRV  
Tomato

**Abstract**

A survey was conducted in Al-Kharj governorate, Riyadh region to identify viruses causing variety of virus-like symptoms on tomato plants. A total of 135 samples were collected from symptomatic tomato plants. Symptoms included mottling, deformation, necrosis of leaves and fruits. Eighteen viruses were tested by DAS-ELISA. *Tomato black ring virus* (TBRV) was the virus of concern as it was not detected in Saudi Arabia before and was detected in 52.6% of the collected samples in this study. RT-PCR was used to confirm detection of TBRV and to sequence the amplified products to determine molecular characteristics of this virus. In the host range test study that was performed using a purified isolate of TBRV, sixteen out of the twenty two tested plants showed symptoms. *Brassica oleracea* was not infected by this virus. Gel electrophoreses (2% agarose) yielded fragments of 978 bp of coat protein gene of TBRV. Nucleotide sequences of purified RT-PCR products for three TBRV Saudi isolates were deposited in the GenBank with the following accession numbers MT274656, MT274657, and MT274658. These isolates of TBRV indicated a close Phylogenetic relationship of (99–100%) among themselves and with five isolates from Poland (95–98%) but a distant relationship of 85% with isolates from England and Lithuania deposited in the GenBank. This is the first report for detection and molecular characterization of TBRV infecting tomato plants in Saudi Arabia.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The first report of *Tomato black ring virus* (TBRV) on tomato plants was in the United Kingdom by Smith in 1946 (Smith, 1946). TBRV belongs to the subgroup B of Genus Nepovirus, Subfamily *Comovirinae*, family *Secoviridae*, order *Picornavirales* (Digiaro et al., 2015; Dunez and Le Gall, 2011). Different strains of this virus cause ring spot diseases of bean, sugar beet, lettuce, raspberry, and strawberry, yellow vein of celery, ‘bouquet,’ pseudo-aucuba of potato, shoot-stunting of peach, one type of mosaic of Robinia pseudoacacia and unnamed diseases of leek and onion, in addition to the black ring of tomato (CABI and EPPO, 1999).

TBRV has three isometric particles (B, M, and T) with a diameter of 26 nm which contain 44%, 32%, and 0% nucleic acid, respectively. TBRV genome consists of two single-stranded RNAs of about 7400nt (RNA1) and 4600nt (RNA2) in length, respectively, carrying a small covalently attached VPg protein at 5′ ends and a poly A tail at their 3′ ends. Each genomic RNA translated to polyprotein, which is cleaved into mature functional proteins by the RNA1-encoded protease. RNA1 is responsible for viral replication and polyprotein processing, while RNA2 carry coat protein and movement genes (Digiaro et al., 2015; Jonczyk et al., 2004b; Mayo and Robinson, 1996).

The first report of a mosaic disease on tomato plants in Saudi Arabia was based only on symptoms in 1957 (Talhouk, 1957). After that, different viruses were detected and identified on tomato using serological and/or molecular methods. The identified viruses included *Tobacco mosaic virus* (TMV), *Tomato yellow leaf curl virus* (TYLCV), *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY), *Tomato mosaic virus* (ToMV), *Tomato spotted wilt virus* (TSWV), *Potato virus X* (PVX), and *Tomato leaf curl virus* (TLCV) (Al-Shahwan et al., 1997; Al-Shahwan, 2003). *Tomato chlorosis virus* (ToCV) and *Tomato leaf curl Sudan virus* (ToLCSDV), which are recently

https://doi.org/10.1016/j.sjbs.2021.01.031
1319-562X/© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
identified in Saudi Arabia (Al-Saleh et al., 2014a; 2014b; Sohrab et al., 2016). Also TBRV was recently reported in Saudi Arabia (Al-Shahwan et al., 2020).

The objectives of this study are to conduct a survey in the Al-Kharj Governorate, Saudi Arabia, to determine the occurrence of TBRV in greenhouse tomatoes, to determine the biological, serological and molecular characteristics of TBRV in Saudi Arabia, and to determine the phylogenetic relationships among the Saudi isolates and isolates reported in other countries.

2. Material and methods

2.1. Sample collection and ELISA test

Hundred thirty-three samples showing virus like symptoms which included: mottling, mosaic, leaf curling, malformation, blisters, necrotic lesions, black streaks, yellowing of the young emerging leaves, vein chlorosis, flower abortion, death of sepals, fruit deformation, fruit spots, and fruit cracking (Fig. 1) were collected from greenhouse grown tomato plants in different seven locations in Al-Kharj Governorate (six samples from Alhazim, twenty-nine from Addubaiyah, forty-four from Alshadidah, seven from Alyamam, twenty-two from Alhayathem, and nineteen from Alhayathem), Riyadh region, during four seasons starting fall 2015. These samples were tested for the occurrence of TBRV and seventeen other viruses normally known to infect tomato namely Alfalfa mosaic virus (AMV), Beet curly top virus (BCTV), Cucumber mosaic virus (CMV), Peanut stunt virus (PSV), Pepino mosaic virus (PepMV), Potato leafroll virus (PLRV), Potato Y virus (PVY), Tobacco etch virus (TEV), Tobacco mosaic virus (TMV), Tomato aspermy virus (TAV), Tomato black ring virus (TBRV), Tomato chlorotic spot virus (TCSV), Tomato mosaic virus (ToMV), Tomato ringspot virus (ToRSV), Tomato spotted wilt virus (TSVV), Tomato yellow leaf curl virus (TYLCV), and Tomato yellow ring virus (TYRV) by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) using specific kits obtained from AC-Diagnostic Inc. (USA). In addition to, two samples were collected from greenhouse grown tomato from Al-Hasa governorate, and were checked for TBRV only.

2.2. TBRV single lesion isolate preparation

The TBRV isolate was biologically purified through a single local lesion technique repeated two times on Chenopodium amaranticolor Coste & Reyn plants (Kahn and Monroe, 1963; Kuhn, 1964). After that, a single local lesion was taken and used to prepare inoculation sap, then greenhouse grown Nicotiana tabacum plant was mechanically inoculated after being dusted with 400 mish carborundum for propagation and maintenance and kept in the greenhouse (25–28 °C). DAS-ELISA test was used to confirm the infection of N. tabacum with the purified TBRV isolate.

2.3. Host range test

Twenty-two plant species belonging to different botanical families were used; which are: Brassica oleracea, C. album, C. amaranticolor, C. glaucum, Citrullus lanatus, Cucumis melo, C. sativus, Cucurbita pepo (zucchini), C. pepo (pumpkin), Datura stramonium, Gomphrena globosa, Lagenaria siceraria, Phaseolus vulgaris, Pisum sativum, N. occidentalis, N. tabacum, N. glutinosa, Solanum lycopersicum, S. melongena, S. nigrum, and S. tuberosum (Brunt et al., 1996). Mechanical inoculation was performed according to a procedure adopted by Hill (1984).

Fig. 1. Some of the virus-like symptoms on greenhouse grown tomato plants observed during sampling. A) Mottling on leaves, B) Necrosis of sepals, C) Fruit deformation, and D) Necrotic blotch on fruits.
Five grams of *N. tabacum* (propagative host) leaves were ground in a mortar and pestle (infected with a single isolate of TBRV), in fifteen ml of 10 mM Potassium Phosphate Buffer, pH 7. Sterile cheesecloth was used to filter the homogenate. Four replicates were used in this experiment, control plants were treated with buffer only. All plants were kept in the greenhouse at a temperature between 25 and 27 °C. Data for symptoms expression were recorded after three weeks postinoculation. The experiment was conducted two times, and DAS-ELISA was used to check TBRV infection with all tested plants (Hill, 1984).

### 2.4. RT-PCR, sequencing and sequence analysis

Total RNA extractions were carried out from nine selected positively DAS-ELISA samples, a total RNA isolation kit (Jena Bioscience, Jena, Germany) was used for this purpose. A pair of primers were designed and used to amplify a fragment of 978 bp in the CP region, F-TRBV: 5'–GAACCAACGCTCTATGGTGCT-3', and R-TRBV: 5'–AGCCCAATCGGAATGGTAGG-3'.

Two-step RT-PCR protocol was used. First-strand complementary DNA (cDNA) was synthesized with specific R-TRBV primer in 20 μl volume using MultiScribe Reverse Transcriptase (High-Capacity cDNA Reverse Transcription Kit, Applied Biosystems™, USA) according to the manufacturer’s instructions. The subsequent PCR in 25 μl volume was performed using one μl RT product as a template cDNA. Amplification was done by using the PCR Master Mix kit from Promega Corporation (Cat no. 75050, Part# 9PIM750) following the manufacturer’s instructions. PCR cycling conditions comprised 2 min of denaturation at 95 °C, then 35 cycles of 95 °C for 30 sec, 65 °C for 45 sec, and 72 °C for 1 min, followed by final cycle at 72 °C for 5 min. Amplified PCR products were then visualized on 1% Agarose Gel as the target DNA size was about 978 bp. The gel was stained with ethidium bromide. PCR products were stored at −20 °C for further use. FavorPrep GEL/PCR Purification mini kit from FAVORGEN BIOTECH CORP. www.favorgen.com was used to purify the PCR products. Purified DNA for the three selected isolates (two from Al-Kharj K9, K41 and one from Alhasa H1 along with specific primers were sent to the Macrogen company, South Korea (Macrogen 10F, 254, Beotkkot-ro, Geumcheon-gu, Seoul, South Korea) for two-directional sequencing. Sequences from the bidirectional sequencing were blasted on the NCBI BLAST program for nucleic acids, and the sequences were identified and compared with other published TBRV sequences on the NCBI website. Sequences were aligned, cleaned by the BioEdit program. These sequences were imported to Lasergene 7 program to check identity percentage of the Saudi isolates of TBRV with each other, and with ten published TBRV isolates on the NCBI website, and then the homology tree was constructed.

### 3. Results

#### 3.1. ELISA results

The available eighteen different ELISA kits mentioned in the material and methods section were used to detect the following viruses: AMV, BCTV, CMV, PSV, PepMV, PLRV, PVY, TEV, TMV, TAV, TBRV, TBSV, TCSV, ToMV, ToRSV, TSWV, TYLCV, and TYRV.

The obtained ELISA results showed that all these viruses were detected in the collected samples in Al-Kharj governorates, Saudi Arabia except PLRV, TBSV, and TSWV. Results showed that 59.4% (79 / 133) of tomato samples (symptomatic samples) were infected with at least one of the detected viruses. Sixty nine out of hundred thirty three samples (51.9%) were found positive to TBRV and out of seventy-nine (87.3%) of DAS-ELISA positively samples were found positive to TBRV, 65.2% were singly infected with TBRV. Whereas 18% of all samples were mixed infection with at least one virus (Table 1). The collected two samples from Al-Hasa were checked by DAS-ELISA for TBRV only and were positive.

#### 3.2. Host range

The host range experiments were done with a pure singly local lesion TBRV isolate (MT174657). Symptoms were observed on sixteen host plants out of the 22 tested plant species. The observed symptoms were local necrotic lesions (*C. amaranticolor* and *Datura stramonium*), Chlorotic local spots (*C. glaucum* and *C. album*). Chlorotic spots develop to systemic chlorosis (*C. quinoa*), mottling (*N. glutinosa*, and *C. pepo*), systemic ring spots and mosaic (*N. tabacum*, *C. globosa*), mosaic (*S. nigrum*, and *P. vulgaris*), stunting and cup-shaped leaf (*S. tuberosum*), mottling and marginal chlorosis (*C. sativus*), mottling and leaf deformation (*S. lycopersicum*), and systemic necrosis on *C. melo* (Fig. 2). Six plant species did not show symptoms. These were *B.oleracea*, *C. lanatus*, *C. pepo* (Pumpkin), *L. sativaria*, *P. sativum*, and *S. melongena*. Five of these plant species (*C. lanatus*, *L. sativaria*, *C. pepo* (Pumpkin), *P. sativum*, and *S. melongena*) were found to be symptomless carrier as they were positive to DAS-ELISA. *B. oleracea* was the only plant species among all host range plants that did not show symptoms and negative to ELISA. It was found that twenty-one out of twenty-two plant species were positive to TBRV by DAS-ELISA (Table 2).

#### 3.3. RT-PCR, sequencing and sequence analysis

cDNA products give smears with all samples, confirming that cDNA was synthesized. Amplification of cDNA gave PCR products, and specific bands were visible with all samples, and their comparison with 50 bp Bioline HyperLadder DNA marker indicated that

| Locations  | Positive DAS-ELISA results |
|------------|----------------------------|
|            | TBRV | AMV | BCTV | CMV | PepMV | PSV | PVY | TAV | TEV | TCSV | TMV | ToMV | ToRSV | TYLCV | TYRV |
| Alhazim    | 6    | 1   | 1    | 0   | 2     | 1   | 1   | 0   | 2   | 4    | 2   | 0    | 0     | 4    | 6    | 0    |
| Addhubahah | 14   | 0   | 0    | 6   | 0     | 0   | 0   | 0   | 2   | 5    | 3   | 0    | 3     | 0    | 7    | 7    |
| Alshaidahd | 29   | 0   | 0    | 0   | 1     | 1   | 0   | 0   | 1   | 2    | 0   | 0    | 2     | 1    | 0    | 4    | 25   |
| Alyamamh   | 3    | 1   | 0    | 0   | 0     | 0   | 0   | 1   | 0   | 0    | 1   | 0    | 0     | 1    | 0    | 1    | 2    |
| Nissah     | 4    | 0   | 2    | 2   | 0     | 2   | 2   | 0   | 0   | 0    | 0   | 0    | 0     | 0    | 2    | 2    |
| Assaha     | 6    | 0   | 0    | 0   | 0     | 0   | 1   | 0   | 0   | 2    | 0   | 0    | 0     | 0    | 2    | 4    |
| Alhayatam  | 7    | 0   | 2    | 0   | 0     | 0   | 0   | 0   | 0   | 0    | 0   | 0    | 0     | 2    | 0    | 5    |
| Total      | 69   | 2   | 5    | 3    | 7     | 3   | 3   | 4   | 1   | 7    | 11  | 5    | 3     | 4    | 4    | 24   | 45   |
| Percentages| 51.9 | 1.5 | 3.8  | 5.3  | 5.2   | 3.7 | 3.8 | 4.0 | 0.8 | 3.8   | 8.3 | 3.8  | 2.3   | 3.0  | 3.0  | 18   | 65.2 |

| Mixed infection | TBRV single infection |
|-----------------|------------------------|
|                 | 18                      |
the size of the specific bands was approximately 978 bp, confirming the presence of TBRV in these samples (Fig. 3).

Sequences of the three Saudi TBRV isolates were submitted to GenBank and assigned the following accession numbers: MT274656 from Al-Hasa, while MT274657, and MT274658 from Al-Kharj. Phylogenetic analyses for the coat protein gene sequence of Saudi and GenBank isolates of TBRV confirmed the percentage similarity between them (Fig. 4). Percentage identity data between all TBRV isolates revealed that all the Saudi isolates were closely related (99–100%), and these isolates were also closely related to isolates from Poland (MG458221 and KX977561) with a percentage of nucleotide similarity of 98–99% (Hasiów-Jaroszewska et al., 2018; Zarzynska-Nowak et al., 2017), and distantly related to isolates from Lithuania (KF678369, and KF678370) and England (X80831.1) (Le Gall et al., 1995; Śneideris and Staniulis, 2014) (Table 3).

4. Discussion

Tomato, one of the most important vegetable crops worldwide suffers infection from several plant pathogens. Several viruses were reported to infect it (Brunet et al., 1990; Hanssen et al., 2010; Jones et al., 2014). Of the 15 viruses detected (out of 18 tested ones) in the samples collected in this study, TBRV was the virus of concern as it was detected for the first time in Saudi Arabia in this investigation and was the most frequently detected virus with a frequency of detection of 52.6%. Although TBRV was discovered on tomato more than six decades ago, scarce reports of its infections were published, and they were mostly on other

![Image of symptoms](image-url)

Fig. 2. The observed symptoms of TBRV on some host range tested plant species: A) Mottling and blisters on *S. lycopersicum*, B) Systemic Ring spots on *N. tabacum*, C) Necrotic spots on *C. amaranticolor*, and D) Chlorotic spots symptoms on *C. quinoa*.

| Plant Species | Symptoms | DAS-ELISA Results |
|---------------|----------|-------------------|
| Chenopodium album | Chlorotic local spots | + |
| Chenopodium amaranticolor | Chlorotic and necrotic local spots | + |
| Chenopodium glaucum | Chlorotic local spots | + |
| Chenopodium quinoa | Chlorotic spots develop to systemic chlorosis | + |
| Datura stramonium | Necrotic local spots | + |
| Gomphrena globosa | Systemic Ring spots and mosaic | + |
| Nicotiana glutinosa | Local chlorotic spots and Mottling | + |
| Nicotiana occidentalis | Tip necrosis | + |
| Nicotiana tabacum | Systemic Ring spots and mosaic | + |
| Solanum lycopersicum | Mottling, leaf deformation | + |
| Solanum nigrum | Mosaic | + |
| Solanum tuberosum | Stunting and cup shaped-like leaves | + |
| Phaseolus vulgaris | Mosaic | + |
| Cucumis sativus | Mottling and marginal chlorosis | + |
| Cucumis melo | Systemic necrosis | + |
| Cucurbita pepo | Mottling | + |
| (zucchini) | | |
| Brassica oleracea | No symptoms | – |
| Citrullus lanatus | No symptoms | + |
| Cucurbita pepo | No symptoms | + |
| (Pumpkin) | | |
| Lagenaria siceraria | No symptoms | + |
| Pisum sativum | No symptoms | + |
| Solanum melongena | No symptoms | + |

Table 2

Symptoms expression of host range plants three weeks after mechanical inoculation with a pure isolate of TBRV under greenhouse conditions, and their response to DAS-ELISA.
crops causing considerable disease incidence and yield losses (Gallitelli et al., 2005; Harrison, 1959; Hollings, 1965) due to its ability of transmission through several methods including mechanical transmission, transmission through seeds, pollen, and two species of nematodes namely *Longidorus attenuatus* and *L. elongatus* (Digiaro et al., 2015; Harrison, 1961; Pospieszny et al., 2020).

Two samples were collected from Al-Hasa Governorate in order to check the spread of TBRV in areas other than Al-Kharj. These samples reacted positively to TBRV, which suggests that this virus enters and spread in Saudi Arabia probably by imported seeds (Johansen et al., 1994; Lister and Murant, 1967; Pospieszny et al., 2020).

The wide host range of this virus and its ability to be transmitted in several ways, probably made it one of the important plant viruses. It is interesting that whereas this virus causes symptomatic infection on plants, it also sometimes causes symptomless infection on other plants (Rymelska et al., 2013). Five of the host range plants in this study were symptomlessly infected with this virus when tested by DAS-ELISA and were positive. All of them were previously reported as hosts for this virus (Bellardi and Pisi, 1985; Brunt et al., 1990; CABI, 2016; Rymelska et al., 2013). Also *B. oleracea* was negative to TBRV by DAS-ELISA which was not reported before as a host for this virus (Table 1).

The polymerase chain reaction (PCR) is a useful method used to detect different plant viruses (Digiaro et al., 2007). TBRV RNA2 has a single open reading frame that encodes polyprotein, which then were cleaved by protease to give the coat protein and movement protein (Demangeat et al., 1992; Digiaro et al., 2015; Jonczyk et al., 2004b; Mayo and Robinson, 1996). Coat protein gene generally used in these types of studies as it was proved as a handy tool for plant virus characterization including Nepoviruses as they were used in this study (Hasiów-Jaroszewska et al., 2015; Śneideris and Stanisius, 2014).

The TBRV population is highly diverse, and the phylogenetic analysis based on the coat protein gene (CP) sequences of the Saudi isolates and other imported NCBI isolates revealed the presence of distinct groups with the nucleotide sequence variability of the CP gene ranging from 0 to 15% similar to what was reported earlier (Jończyk et al., 2004a; Pospieszny et al., 2020; Śneideris and Stanisius, 2014). CP gene nucleotide sequence comparisons show that all Saudi TBRV isolates are located in the same clade of the Polish isolates while it located in a different clade for England isolate and that are in a cluster in a different clade of Lithuanian isolates (Le Gall et al., 1995; Śneideris and Stanisius, 2014).

### Table 3

| Accession | Country       | Host              | MT274656 | MT274657 | MT274658 |
|-----------|---------------|-------------------|----------|----------|----------|
| MT274656  | Saudi Arabia  | *S. lycopersicum* | 100      | 100      | 99       |
| MT274657  | Saudi Arabia  | *S. lycopersicum* | 100      | 100      | 99       |
| MT274658  | Saudi Arabia  | *S. lycopersicum* | 99       | 99       | 100      |
| MG458221  | Poland        | *S. lycopersicum* | 98       | 98       | 97       |
| KG977561  | Poland        | *S. lycopersicum* | 98       | 98       | 97       |
| KR139951  | Poland        | *S. tuberosum*    | 96       | 96       | 95       |
| KR139952  | Poland        | *Cucumis sativus* | 95       | 95       | 94       |
| KT923164  | Switzerland   | –                 | 94       | 95       | 95       |
| AY157994  | Poland        | *R. pseudoacacia* | 94       | 95       | 95       |
| KF678372  | Lithuania     | *Clematis sp.*    | 88       | 88       | 88       |
| KF678370  | Lithuania     | *Lamprocapnos spectabilis* | 85 | 86 | 86 |
| KF678369  | Lithuania     | Hosta sp.         | 85       | 85       | 85       |
| X08031    | England       | *Daucus carota*   | 84       | 85       | 85       |
5. Conclusions

TBRV was detected for the first time in greenhouse tomato plants grown in Saudi Arabia. Some plant species react silently to TBRV infection without expressing any symptoms which may consider as a source of infection in the greenhouse. Since TBRV was detected in Al-Kharj Governorate in Riyadh Region, and in Al-Hasa Governorate in the Eastern Region the rest of the Kingdoms regions ought to be surveyed to determine the extent of this virus spread in the country. The suggested survey will be more useful if it also includes hosts other than tomato such as grape, potato, strawberry, and other hosts in the Kingdom of Saudi Arabia.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors extend their sincere appreciation to the Deanship of Scientific Research, King Saud University for supporting the work. No financial support has been received for this study.

Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent was obtained from all individual participants included in the study.

Authors contributions

A. Al-Shudifat, I. Al-Shahwan, and M. Al-Saleh: samples collection and conducting the experiments. A. Al-Shudifat, O. Abdalla, and M. Amer: analysis and interpretation of results. A. Al-Shudifat, I. Al-Shahwan, and O. Abdalla: draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

References

Al-Saleh, M., Brown, J.K., Idris, A.M., 2014. Two begomoviral species causing yellow leaf curl disease of tomato: distinct spatial distribution patterns in the Arabian Peninsula. in: Poster Session: Molecular/Cellular/Plant-Microbe Interactions- Proteomics/Metabolomics/Genomics. p. 478.
Al-Saleh, M.A., Al-Shahwan, I.M., Shakeel, M.T., Amer, M.A., Orfanidou, C.G., Katis, N., I., 2014b. First report of Tomato chlorosis virus (ToCV) from Gladiolus. Riv. di Ortoflorofruttic. Ital. 69, 299–309.
Brunt, A., Crabtree, K., Gibbs, A., 1990. Viruses of tropical plants: descriptions and species-specific primers for the differential and simultaneous RT-PCR detection of grapevine-infecting nepoviruses of subgroups A B and C. J. Virol. Methods 141, 34–40.
Djigaro, M., Elborno, I., Martelli, G.P., 2007. Development of degenerate and species-specific primers for the differential and simultaneous RT-PCR detection of grapevine-infecting nepoviruses of subgroups A B and C. J. Virol. Methods 141, 34–40.
Djigaro, M., Al-Shahwan, I.M., Al-Saleh, M.A., Elborno, I., 2015. The sequencing of the complete genome of a Tomato black ring virus (TBRV) and of the RNA2 of three Grapevine chrome mosaic virus (GCMV) isolates from grapevine reveals the possible recombinant origin of GCMV. Virus Genes 50, 165–171.
Duney, J., 2011. Nepovirus: In: Tidona, C., Darai, G. (eds). The Springer Index of Viruses. New York, pp. 361–369.
Galitelli, D., Rana, G.L., Vovlas, C., Martelli, G.P., 2005. Viruses of globe artichoke: an overview. Acta Hort. 681, 553–572.
Hansen, I.M., Lapidor, M., Thomma, B.P.H.J., 2010. Emerging viral diseases of tomato crops. Mol. Plant-Microbe Interact. 23, 539–548.
Harrison, B.D., 1961. Transmission of a Strain of Tomato Black Ring Virus by Longidorus elongatus (Nematoda) 485, 480–485.
Harrison, B.D., 1959. The Pattern of Field Infection of Potato By the Beet Ringspot Strain of Tomato Black Ring, a Soil-Borne Virus. Ann. Appl. Biol. 47, 557–564.
Hasiò-Jaroszewska, B., Budzęska, D., Borodynko, N., Pospieszny, H., 2015. Rapid detection of genetically diverse tomato black ring virus isolates using reverse transcription loop-mediated isothermal amplification. Arch. Virol. 160, 3075–3078.
Hasiò-Jaroszewska, B., Minicja, J., Zary´ zyska-Nowak, A., Budzęska, D., Elena, S.F., 2018. Defective RNA particles derived from Tomato black ring virus genome interfere with the replication of parental virus. Virus Res. 250, 87–94.
Hill, S.A., 1984. Methods in plant virology / Stephen A. Hill, Methods in plant virology; v. 1. Published on behalf of the British Society for Plant Pathology by Blackwell Scientific Publications, Oxford [Oxfordshire]; Boston.
Hollings, M., 1963. Some properties of celery yellow vein, a virus serologically related to tomato black ring virus. Ann. Appl. Biol. 55, 459–470.
Jonczyk, M., Borodynko, N., Pospieszny, H., 2004a. Restriction analysis of genetic variability of Polish isolates of Tomato black ring virus. Acta Biochim. Pol. 51, 673–681.
Jonczyk, M., Le Gall, O., Patucha, A. Borodynko, N., Pospieszny, H., 2004b. Cloning and sequencing of full-length cDNAs of RNA1 and RNA2 of a Tomato black ring virus isolate from Poland. Arch. Virol. 149, 799–807.
Jonczyk, M., Le Gall, O., Patucha, A. Borodynko, N., Pospieszny, H., 2004b. Cloning and sequencing of full-length cDNAs of RNA1 and RNA2 of a Tomato black ring virus isolate from Poland. Arch. Virol. 149, 799–807.
Kahn, R.P., Monroe, R.L., 1963. Detection of tobacco veinal necrosis strain of potato virus Y in Solanum calendulaceum and S. andigenum introduced into United States. Phytopathology 53, 1356.
Kahin, R.P., 1964. Separation of cowpea virus mixtures. Phytopathology. 54, 739.
Kahin, R.P., Monroe, R.L., 1963. Detection of tobacco veinal necrosis strain of potato virus Y in Solanum cardenasii and S. andigenum introduced into United States. Phytopathology 53, 1356.
Kahin, R.P., 1964. Separation of cowpea virus mixtures. Phytopathology. 54, 739.
Kahin, R.P., Monroe, R.L., 1963. Detection of tobacco veinal necrosis strain of potato virus Y in Solanum cardenasii and S. andigenum introduced into United States. Phytopathology 53, 1356.
Kahin, R.P., 1964. Separation of cowpea virus mixtures. Phytopathology. 54, 739.
Kahin, R.P., Monroe, R.L., 1963. Detection of tobacco veinal necrosis strain of potato virus Y in Solanum cardenasii and S. andigenum introduced into United States. Phytopathology 53, 1356.
Kahin, R.P., 1964. Separation of cowpea virus mixtures. Phytopathology. 54, 739.
Kahin, R.P., Monroe, R.L., 1963. Detection of tobacco veinal necrosis strain of potato virus Y in Solanum cardenasii and S. andigenum introduced into United States. Phytopathology 53, 1356.
Kahin, R.P., 1964. Separation of cowpea virus mixtures. Phytopathology. 54, 739.
Kahin, R.P., Monroe, R.L., 1963. Detection of tobacco veinal necrosis strain of potato virus Y in Solanum cardenasii and S. andigenum introduced into United States. Phytopathology 53, 1356.
Kahin, R.P., 1964. Separation of cowpea virus mixtures. Phytopathology. 54, 739.
Kahin, R.P., Monroe, R.L., 1963. Detection of tobacco veinal necrosis strain of potato virus Y in Solanum cardenasii and S. andigenum introduced into United States. Phytopathology 53, 1356.
Kahin, R.P., 1964. Separation of cowpea virus mixtures. Phytopathology. 54, 739.
Kahin, R.P., Monroe, R.L., 1963. Detection of tobacco veinal necrosis strain of potato virus Y in Solanum cardenasii and S. andigenum introduced into United States. Phytopathology 53, 1356.
Kahin, R.P., 1964. Separation of cowpea virus mixtures. Phytopathology. 54, 739.