



EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA
PROTECTION DES PLANTES

Pest Risk Analysis for

Tomato mottle mosaic virus (Tobamovirus)



Sui *et al.*, 2017a - brown necrotic lesions on leaves of tomato with Tm-2² gene

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The risk assessment follows EPPO standard PM 5/5(1) *Decision-Support Scheme for an Express Pest Risk Analysis* (available at <http://archives.eppo.int/EPPOStandards/prah.htm>), as recommended by the Panel on Phytosanitary Measures. Pest risk management (detailed in **Erreur ! Source du renvoi introuvable.**) was conducted according to the EPPO Decision-support scheme for quarantine pests PM 5/3(5). The risk assessment uses the terminology defined in ISPM 5 *Glossary of Phytosanitary Terms* (available at <https://www.ippc.int/index.php>).

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The Working Party on Phytosanitary Regulation decided to not recommend the addition of tomato mottle mosaic virus (ToMMV) to the List of pests recommended for regulation for the time being, and therefore to not propose risk management options. The Working Party supported the recommendations that more surveys and a better understanding of the current situation in the EPPO region should be sought. More information on the testing to be used in that framework will be collected by EPPO, before the outcome of the Euphresco project is available. Possible topics for research are reported in section 18.

The reasons for not recommending ToMMV for regulation were in particular:

- ToMMV is possibly more widespread in the EPPO region than currently known, and the situation should be verified. In particular, it has recently been detected in a large seed bank.
- ToMMV is similar to tomato mosaic virus (ToMV), which is present in many EPPO countries and is not regulated in most. Before it was described in 2013, ToMMV may have been misidentified as ToMV.
- Finally, *Tm-2²* genes in tomato provide some resistance (no clear demonstration of resistance breaking) and it is also probably the case with *L*-genes/alleles in pepper.

Pest Risk Analysis for *Tomato mottle mosaic virus (Tobamovirus)*

PRA area: EPPO region

Prepared by: Expert Working Group (EWG) on tomato mottle mosaic virus

Date: 22-25 February and 8-10 March 2022. Further reviewed and amended by EPPO core members and Panel on Phytosanitary Measures (2022-05, see below).

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The first draft of the PRA was prepared by Fabienne Grousset, consultant for EPPO. Nataša Mehle and Zhibo Hamborg conducted a targeted review of an early draft.

Personal communications in this PRA were obtained in November 2021- April 2022 from: S. Adkins (USDA-ARS, Florida, USA), D. Allen (DEFRA, UK - EWG member), M. Botermans (NVWA, The Netherlands); J. Bustová (Central Institute for Supervising and Testing in Agriculture, Czech Republic), B. Geraats (Nunhems Netherlands, Nunhem Netherlands), V. Grimault (Groupe d'Étude et de contrôle des Variétés Et des Semences

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The EWG (and accordingly its composition) was planned as a face-to-face meeting but eventually met during videomeetings in 22-25 February and 8-10 March in accordance with the Covid arrangements agreed for PRA EWGs.

Ratings of likelihoods and levels of uncertainties were made during the meeting. These ratings are based on evidence provided in the PRA and on discussions in the group. Each EWG member provided a rating and a level of uncertainty anonymously and proposals were then discussed together in order to reach a final decision. Such a procedure is known as the Delphi technique (Schrader *et al.*, 2010).

Following the EWG, the PRA was further reviewed by the following core members: J. Boberg, J.M. Guitian Castrillon, S. Hannunen, F. Petter, C. Picard, G. Schrader, R. Tanner, N. Üstün; as well as by Š. Linhartová (Central Institute for Supervising and Testing in Agriculture, National Reference Laboratory, Czech Republic).

The PRA, in particular the conclusion (section 15) and section on risk management (section 16), was reviewed and amended by the EPPO Panel on Phytosanitary Measures on 2022-05-13. EPPO Working Party on Phytosanitary Regulation and Council agreed that, for the time being, ToMMV should not be added to the A2 List of pests recommended for regulation as quarantine pests.

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Summary of the Pest Risk Analysis for tomato mottle mosaic virus (Tobamovirus)

PRA area: EPPO region (Albania, Algeria, Austria, Azerbaijan, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Guernsey, Hungary, Ireland, Israel, Italy, Jersey, Jordan, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Luxembourg, Macedonia, Malta, Moldova, Montenegro, Morocco, Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Tunisia, Turkey, Ukraine, United Kingdom, Uzbekistan).

Describe the endangered area: Tomato mottle mosaic virus (ToMMV) could establish in the whole EPPO region wherever hosts are grown, i.e. throughout the EPPO region in protected conditions, and outdoors south of ca. 63°N.

The evidence suggests that economic impact is most likely on tomato, but with more impact where cultivars that do not carry the *Tm-2²* resistance gene are used. There may also be economic impact on *Capsicum*, wherever grown, but impact may be limited to cultivars that do not carry the *L* resistance genes/alleles. There are uncertainties about the potential impact (see section 13), particularly linked to the effectiveness of the *Tm-2²* and *L* genes/alleles and the extent to which tomato and *Capsicum*, cultivars are used with these genes/alleles in the EPPO-region.

Main conclusions: A few factors (further developed in section 15) affected different stages of the assessment:

- The use of resistant cultivars (throughout this summary, resistant cultivars mean carrying the *Tm-2²* resistance gene for tomato, and *L* genes/alleles (*L1*, *L3* or *L4*) for *Capsicum* is considered to reduce ToMMV occurrence, infection and damage. Resistant cultivars are widely used especially in intensive commercial fresh fruit production of tomato and *Capsicum* in protected conditions (at least in the EU). This may also be the case in outdoor crops for fresh fruit production, but not for tomato cultivars for processing grown in the field. Information was not available for all EPPO countries.
- ToMMV may have a wider distribution than currently reported (including in EPPO countries). It may have arrived already many times on imported seeds. ToMMV-specific tests have not been commonly used in the past, and outbreaks of ToMMV could have been thought to be tomato mosaic virus (ToMV), and therefore not reported.
- Although pea, common bean and tobacco are hosts, it was not possible to cover them in several steps of the PRA due to lack of information.

Entry: The likelihood of entry was considered as high with a moderate uncertainty, the highest ratings being seeds of tomato and *Capsicum*, and plants for planting (except seeds and pollen) of tomato and *Capsicum*. Transfer is less likely if the cultivar carries the resistance genes above. However a small portion of the plants may still become infected (see section 2.7).

Establishment: The likelihood of establishment of ToMMV was rated high for outdoors in the EPPO region where host plants are grown (with a moderate uncertainty). Under protected conditions, the likelihood of establishment was assessed to be moderate (with a moderate uncertainty), because of the wide use of resistant cultivars, and because establishment in protected conditions does not appear to have happened to date.

The magnitude of spread was rated moderate with a high uncertainty. The pest could spread locally by natural dispersal within a production area or more widely via human-assisted mechanical transmission by workers, visitors, tools and equipment as well as with the trade of plants for planting, seed and fruit.

Impact (economic and social) was rated moderate with a moderate uncertainty. Fresh fruit production (in protected crops or in the field) is more likely to be affected, as well as sites producing hybrid seed in the field. However, impact on tomato and *Capsicum* will depend mostly on the susceptibility of cultivars that are being grown in EPPO countries. Resistant cultivars may not be used in all types of crops and throughout EPPO countries. The direct impact (yield losses, additional crop protection costs) may be low in areas where nearly all tomato crops carry the *Tm-2²* resistance gene and not different from that of ToMV. However, while a real resistance break has not been shown to date, in one case, ToMMV was reported to be the causal agent for a disease outbreak on tomato cultivars with the *Tm-2²* gene, and there is some evidence that high temperatures may help to cause expression of symptoms in some *Tm-2²* cultivars.

The EWG noted that information is lacking regarding the current situation of ToMMV in the EPPO region (see sections 6 and 15). The EWG recommended that ToMMV-specific testing is performed on field samples and seed banks to verify the situation. This recommendation and others are detailed in section 18.

There was no agreement in the EWG regarding whether ToMMV should be recommended for regulation. Arguments are developed in section 15. The PPM decided to not recommend the addition of ToMMV to the List of pests recommended for regulation for the time being. However, it stressed the importance of gathering more information and took note of possible topics for research in section 18. The Panel commented that more guidance for testing would be valuable and recommended that additional surveillance is performed in the EPPO region.

Phytosanitary risk for the <i>endangered area</i> (Individual ratings for likelihood of entry and establishment, and for magnitude of spread and impact are provided in the document)	High <input type="checkbox"/>	Moderate <input checked="" type="checkbox"/>	Low <input type="checkbox"/>
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Level of uncertainty of assessment (see Q 17 for the justification of the rating. Individual ratings of uncertainty of entry, establishment, spread and impact are provided in the document)	High <input type="checkbox"/>	Moderate <input checked="" type="checkbox"/>	Low <input type="checkbox"/>
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Other recommendations: Recommendations on testing to clarify the situation in the EPPO region and other recommendations are developed in section 18.

Stage 1. Initiation

Reason for performing the PRA:

Tomato mottle mosaic virus (ToMMV, Tobamovirus) was first described in 2013 infecting tomato (*Solanum lycopersicum*) crops in Mexico. It was subsequently found in the Americas, Asia and Europe causing infections on tomato and pepper (*Capsicum* spp.)¹. A few other natural hosts in the family Solanaceae, Leguminosae and Chenopodiaceae have also been identified. ToMMV is a relatively recently identified tobamovirus which presents similarities with another tobamovirus, tomato brown rugose fruit virus that causes high yield losses in tomato crops (ToBRFV - EPPO A2 List). ToMMV was added to the EPPO Alert List in 2020 as recommended by the Panel on Phytosanitary Measures (PPM) (EPPO, 2020a). The PPM selected ToMMV as a possible priority for PRA in 2021, and the Working Party for Phytosanitary Regulations selected it for PRA in June 2021.

The EPPO standard PM 5/5 [Decision-Support Scheme for an Express Pest Risk Analysis](#) was used, as recommended by the PPM.

The EPPO PRA on ToBRFV was extensively used (EPPO, 2020b) when developing this PRA. **Blue text in the present PRA is from the PRA on ToBRFV (except that ‘ToBRFV’ is replaced by ‘ToMMV’ where relevant). When text was adjusted from the PRA on ToBRFV, the changes are marked in black (deletions are not indicated). Consideration of pest risk management is based on the PRA for ToBRFV (EPPO, 2020b) and is not repeated in the present PRA (see section 16).**

National PRA/quickscans on this virus were available from Germany (JKI, 2020), the Netherlands (Dutch NPPO, 2020) and UK (UK NPPO, 2021; unpublished).

PRA area: EPPO region in March 2022 (map at https://www.eppo.int/ABOUT_EPPO/eppo_members)

Stage 2. Pest risk assessment

1. Taxonomy

Taxonomic classification. Kingdom: Viruses and Viroids; Classification: Viruses; Family: *Virgaviridae*; Genus: *Tobamovirus*; Species: *Tomato mottle mosaic virus* (ToMMV)

Spanish name: virus del mosaico moteado del tomate (Nolasco-García *et al.*, 2020).

Portuguese name: vírus do mosaico marmoreado do tomateiro (EPPO, 2022).

Note that the acronym ToMMV is also used in the literature for a begomovirus, tomato mild mosaic virus.

The 2020 ICTV (International Committee on Taxonomy of Viruses) guidelines on virus taxonomy have been followed for the capitalisation and the italicisation of the virus and disease name (ICTV, 2020)

There is a high amino-acids sequence similarity between tobacco mosaic virus (TMV), tomato mosaic virus (ToMV) and ToMMV, and infections diagnosed as TMV or ToMV using serological methods might actually be ToMMV (Zhan *et al.*, 2018).

A number of isolates from Brazil, Iran and China deposited in Genbank as ToMV are now considered to correspond to ToMMV (details in Annex 1). Some records extend the host range and the distribution of ToMMV (reflected in sections 6 & 7). These isolates are still under the name ToMV in Genbank, and this PRA clearly mentions when information relates to them. A NCBI blast² analysis with all isolates of Annex 1 with check of the highest similarity (all hits with more than 90% similarity), showed that no other isolates of ToMMV has been uploaded to NCBI as ToMV (as of 1 February 2022).

¹ Note that this PRA uses both terms, pepper and *Capsicum*.

² National Center for Biotechnology Information [BLAST: Basic Local Alignment Search Tool \(nih.gov\)](#)

It is likely that ToMMV has been (mis)identified as ToMV in more cases, such as prior to its description in 2013, or in the case of ToMV-positive samples obtained through serological (e.g. ELISA) tests. A wider presence of ToMMV is also supported by the recent finding of ToMMV in tomato and pepper seed collections in the Netherlands (see section 6).

EPPO code: TOMMV0

2. Pest overview

Limited scientific data have been published on ToMMV to date.

When data are missing, non-published data and data on other scientifically well-researched tobamoviruses such as ToMV, ToBRFV, TMV and cucumber green mottle mosaic virus (CGMMV) have been used to provide information for the present assessment of the risk of ToMMV.

2.1 Morphology

Tobamoviruses are rod-shaped particles of 18 nm in diameter and have a predominant length of 300–310 nm. They consist of a single-stranded RNA-molecule of 6.3 to 6.6 kb, arranged in four open reading frames, that is located in a crinkled cylindrical capsid (ICTV, 2019).

2.2 Transmission

Transmission from soil contaminated with infested plant residues

In experiments, healthy seeds of tomato and *Capsicum* were sown in soil containing ToMMV (in which ToMMV-infected plants had been cultivated). ToMMV was detected in approximately 80% of the tomato and *Capsicum* seedlings (Li *et al.*, 2020). This percentage is very high compared to current knowledge of such transmission for tobamoviruses, and there are not enough details in the article on how the testing was conducted to be certain about the final results (i.e. that all positively tested plants had been infected through soil-borne inoculum). No information was found for other hosts.

► ToMMV may be present in soil and growing media if infested plant material has been in contact with them. Transfer to the growing host plant may be possible through such soil contaminated with infested plant residues.

Seed-borne transmission

ToMMV was detected in many seed lots of tomato and *Capsicum* (e.g. Australian Government, 2019; Lovelock *et al.*, 2020; Tiberini *et al.*, 2022; Mut Bertome, 2021). Ling (2021) noted that ToMMV is assumed to be seed-borne in a similar manner to TMV and ToMV, but further study is needed to confirm this. There is currently no biological evidence of seed-borne transmission of ToMMV.

The rate of transmission of a tobamovirus through natural germination of contaminated seeds is usually low (Dombrovsky & Smith, 2017). However, due to ease of mechanical transmission, the virus can spread rapidly through a crop starting from a few infected plants, through handling.

The viral particles of tobamoviruses are extremely stable and infectivity is preserved in seeds for up to several years (Dombrovsky & Smith, 2017). Most of the tobamoviruses contaminate the seed coat, but not the embryo (Dombrovsky, pers. comm., 2019 cited in EPPO, 2020b). However, *in situ* immunofluorescence analysis of CGMMV reveals that the perisperm-endosperm envelope is also contaminated by this tobamovirus (Dombrovsky & Smith, 2017). ToBRFV is “localized in the external teguments of the seed, although in some cases, probably depending on the viral accumulation that plays an important role in the transmission, it seems to be found in the endosperm, but never in the embryo” (Davino *et al.*, 2020). Similarly, association with the endosperm has not been excluded for ToMMV.

Under experimental conditions, Li *et al.* (2020) showed transmission from seed to seedlings in *N. tabacum* var. Xanthi inoculated with ToMMV: a total of 122 seedlings were tested, 48 of which were found to be infected. However this rate of seed to seedling transmission is very high and there are not enough details in

the article on how testing was conducted to be certain about the final results. No information is currently available on transmission for tomato, *Capsicum* or other hosts.

► **ToMMV can be associated with seeds (at least for tomato, *Capsicum*). Although there is currently no biological evidence of seed-borne transmission of ToMMV, based on information available for other tobamoviruses, ToMMV is assumed to be seedborne in a similar manner as TMV and ToMV.**

Mechanical transmission

Tobamoviruses are also mechanically transmitted from plant to plant through common cultural practices causing wounds or microlesions (e.g. hands, clothes, tools including knives, equipment including trellising ropes, movement of tractors and other machinery in production fields) (Broadbent, 1976; Dombrovsky & Smith, 2017 cited EPPO, 2020b).

In transmission experiments, ToMMV could be transmitted by mechanical inoculation (both sap inoculation and injection into leaves) (Ambros *et al.*, 2017; Li *et al.*, 2020; Sui *et al.*, 2017a).

Aerial irrigation (e.g. when using sprinklers) can cause injury to plants that can facilitate infection. This has been demonstrated for CGMMV (Dombrovsky *et al.*, 2015). No information was available for ToMMV.

Although no direct experiment has been conducted on grafting for ToMMV transmission, most likely it can be transmitted through the grafting process, based on other tobamoviruses.

► **From evidence available, it appears that ToMMV can easily be transmitted mechanically, similarly to other tobamoviruses.**

Water-mediated transmission

No study was found on the possible transmission of ToMMV with circulating water as a source of root infection, but it is considered a likely pathway for spread. Several studies have found viruses, including tobamoviruses, associated with water. For example, in Slovenia, ToMV was found in rivers, gravel pit and in a sample of irrigation water from a ToMV-infested greenhouse (Boben *et al.*, 2017). Bačnik *et al.* (2020) found that pepper mild mottle virus (PMMoV), ToMV and tobacco mild green mosaic virus (TMGMV) remain infective even after conventional wastewater treatment. Infectious ToMV and TMV were found in irrigation and drainage canals (Jeżewska *et al.*, 2018). Infective tobamoviruses have been shown to survive in nutrient solution for up to six months (Pares *et al.*, 1992). In experiments in a hydroponic system, ToMV could be released from infested tomato plant roots, survive in a nutrient solution, and infect other tomato plants through roots, without root contact, and produce symptoms (Pares *et al.*, 1992). Finally, water-mediated transmission of CGMMV was shown by Li *et al.* (2016) (cited in Filipić *et al.*, 2021).

► **Water-mediated transmission is likely, such as with circulating water.**

Transmission by bumblebees

Tobamoviruses have no known natural vectors (Adams *et al.*, 2016). Okada *et al.* (2000, cited in Levitzky *et al.*, 2019) showed that foraging bumblebees can transmit TMV from infected to non-infected tomato plants adjacent to each other in a greenhouse (with a primary inoculum present in the greenhouse). Levitzky *et al.* (2019) demonstrated that transmission of ToBRFV is possible via bumblebee colonies (*Bombus terrestris*) used for pollination. ToBRFV may adhere to the pollen grains attached to the bumblebees or be present in crude sap on their bodies and mandibles. The bumblebees may transmit the virus by causing wounds when using their mandibles to grasp the anther cone, or microlesions when vibrating bodies (Levitzky *et al.*, 2019; Velthuis & van Doorn, 2006).

► **No specific information was found for ToMMV, but bumblebees may possibly transmit the virus.**

Pollen transmission

Some tobamoviruses may be pollen transmitted in the absence of pollinators, such as CGMMV (Liu *et al.*, 2014). However, this report is not confirmed outside of experimental conditions.

► **There is no evidence of pollen transmission of ToMMV.**

2.3 Survival outside of living host plants

Tobamoviruses can survive outside of a host on inert (e.g. cardboard, pallet, transport material, tools, clothes, vehicles, stakes) and biological surfaces (e.g. human hands, plants remnants, pollinators insects) as well as in nutrient film solutions and soil for months without losing their virulence (Li *et al.*, 2016; Smith *et al.*, 2019). No specific data were found for ToMMV; data for other species are provided in the EPPO PRA on ToBRFV (EPPO, 2020b).

No specific data were found regarding survival of ToMMV in soil, but the virus is expected to survive for some time. In dry soil, powdered leaf debris can remain infective with ToMV for two years, but in moist soil, leaf debris loses infectivity within one month. ToMV survives in fallow soil for 22 months in root debris at a depth of 120 cm and for longer than two years when carried under black plastic covered with a layer of clean compost (APS, 2014). Survival of ToBRFV in leaf debris in soil depends on the type of soil and moisture content which gives an indication of the level of microbial activity and other environmental conditions to break down the ToBRFV infectivity in the decayed tissue (Dombrovsky, unpublished data, 2019 cited in EPPO, 2020b).

As part of experiments conducted in the UK, it was shown that ToBRFV can survive for at least six months on some surfaces (including hard plastic, polythene and glass), at least three months on stainless steel, up to three months on concrete, at least four weeks on aluminium, and at least two hours on skin and gloves (and still cause infection) (<https://ahdb.org.uk/knowledge-library/tomato-brown-rugose-fruit-virus-survival-and-disinfection>).

Tobamoviruses may be found in extreme conditions: ToMV can be present at high altitude, in fog of an arid area, in spring water as well as in cigarettes and in the mouth of a person smoking (Castello *et al.*, 1999, 1995). TMV has also been found associated with cigarettes and the saliva of smokers: in a study on 47 cigarettes from different brands, TMV was found in all cigarettes, and was viable in 53% of them (Balique *et al.*, 2012). Other tobamoviruses such as pepper mild mottle virus (PMMoV) are known to be used as indicators of water quality (Kitajima *et al.*, 2018). PMMoV has been shown to survive the harsh environment of the human gastrointestinal tract, and to remain infectious even after excretion in faeces (Zhang *et al.*, 2006). Finally, ToMMV was detected in the faeces of an infant (<1 year) in Mexico (Aguado-García *et al.*, 2020).

2.4 Temperature requirements

ToMMV has been found both outdoors and in protected conditions.

Tobamoviruses can overwinter in soil (Smith *et al.*, 2019). For example, CGMMV was shown to overwinter in soil sample with debris of infected plant at a depth between 10 and 30 cm with an average air temperature of 1°C and a minimum air temperature of -9°C without losing its virulence (Li *et al.*, 2016).

There are no published experiments assessing the survival of ToMMV at very low or high temperatures in soil, growing media or other material (e.g. surfaces in the greenhouses). Noble and Roberts (2004) mention that several plant viruses are temperature-tolerant (CGMMV, PMMoV, tobacco rattle virus, ToMV and TMV). TMV requires a peak compost temperature above 68°C and a composting period longer than 20 days for eradication. However, TMV is degraded in compost over time, and can be eradicated after a composting period of 26 weeks, even at low temperature (31°C). ToMV in infected seeds can withstand over 70°C in an incubator for over 20 days. Recent research conducted at Fera (GB) on ToBRFV suggests that treatment of trays in a waterbath at 70°C for five minutes is ineffective, but 90°C will inactivate the virus (<https://ahdb.org.uk/knowledge-library/tomato-brown-rugose-fruit-virus-survival-and-disinfection>). Follow-up trials in early 2021 confirmed that 90°C is the thermal inactivation point for ToBRFV, and that it is the action of heat rather than a washing process that is required to ensure the virus is killed (AHDB, 2022).

2.5 Nature of the damage

ToMMV is reported to decrease the production of infected plants (Turina *et al.*, 2016). Tomato fruits can become unmarketable (Maudarbaccus *et al.*, 2021; Sui *et al.*, 2017a).

2.6 Symptoms of infection

As for other tobamoviruses, symptoms can vary depending on factors such as co-infection with other viruses, plant species, cultivar and environmental conditions (for details, see EPPO, 2020b).

The following symptoms of ToMMV are reported in the literature:

On tomato:

- plants: stunting (Ambrós *et al.*, 2017, Sui *et al.*, 2018 in experiments), complete loss of flowers is observed when young plants were infected (and therefore no fruit production) (Sui *et al.*, 2017a).
- shoots: chlorosis (Maudarbaccus *et al.*, 2021).
- leaves: mosaic, mottling, necrosis/necrotic spots, chlorosis, crinkling, blistering, epinasty, deformation, leaf curl (Ambrós *et al.*, 2017; Turina *et al.*, 2016; Li *et al.*, 2013; ICAR-IIHR, 2017; Webster *et al.*, 2014; Zhan *et al.*, 2018; Sui *et al.*, 2017a; Li *et al.*, 2017; Maudarbaccus *et al.*, 2021). Li *et al.* (2013) mention that mature plants present mosaic and deformation while inoculated seedlings present rapid tissue necrosis on the upper leaves. In experiments, Tu *et al.* (2021) also reported for the first-time enations along the blade margin on the undersides of the leaves.
- fruits: necrotic lesions and fruit necrosis (Sui *et al.*, 2017a). Uneven ripening and necrotic spots on fruits (Maudarbaccus *et al.*, 2021).

Symptoms on tomato are illustrated in Annex 2.

On Capsicum:

- plants: fast apical yellowing and necrosis (observed on *C. annuum*; Ambrós *et al.*, 2017), stunting (observed on *C. annuum* var. *grossum*; Li *et al.*, 2017)
- leaves: mottle, shrinking, necrosis (Li *et al.*, 2014), crinkling, mosaic, necrosis on seedlings (Zhan *et al.*, 2018); chlorosis, mosaic, necrosis (observed on *C. frutescens*, Yunnan, isolate YYMLJ) and mottle, necrosis (*C. annuum* var. *grossum* in Lhasa, TiLhaLJ) (Li *et al.*, 2017).
- fruit: no symptoms reported.

On Phaseolus vulgaris: chlorosis and blistering on leaves (Alavi & Massumi, 2014; Fig 1). Mottle and necrosis in inoculation experiments (Li *et al.*, 2020).

On Pisum sativum: foliar chlorosis, mosaic, malformation and necrosis symptoms (Zhang *et al.*, 2021).

On Chenopodium murale: asymptomatic (see details in section 7).

Experimental hosts: similar symptoms as above are described in the literature available, and are listed in Annex 3. For some species, there are conflicting results concerning the experimental host status; details are provided in section 7, Table 2.

In most host species used in inoculation tests (incl. tomato and *Capsicum*), symptoms of ToMV and ToMMV were indistinguishable, including necrosis, chlorosis, mosaic, leaf distortion, plant stunting (Sui *et al.*, 2017a).

Typical symptoms of hypersensitivity are local necrotic lesions.

2.7 Resistance

Tomato (*Solanum lycopersicum*)

In tomato, resistance genes (*Tm-1*, *Tm-2/Tm-2²*) have been identified that provided resistance to several tobamoviruses such as ToMV and TMV. These genes have been crossed from wild tomato germplasm into cultivated tomato varieties and are currently used for protection in most commercial cultivars (APS, 2014; Luria *et al.*, 2017). While the resistance provided by *Tm-1* and *Tm-2* was temporary due to emergence of new pathotypes of ToMV, the resistance of *Tm-2²* has remained effective against ToMV. Thus *Tm-2²* is widely used in tomato breeding (García-Arenal & McDonald, 2003).

Real resistance-breaking of *Tm-2²* has been observed for ToBRFV, but this is not the case for ToMMV. In certain cases, a number of plants of *Tm-2²* cultivars of tomato have been found infected by ToMMV in experiments or in the field, as reported below:

- In resistance tests in China, Li *et al.* (2020) found different degrees of susceptibility/resistance amongst 10 tomato varieties. However, the article does not specify whether these tomato varieties were harbouring resistance genes. It was not possible to obtain further information.
- In Brazil, Nagai (2017) reported that a tomato cultivar containing *Tm-2²* resistance gene did not exhibit symptoms of ToMMV infection after experimental inoculation. ToMMV was not detected in any plants by serology. When testing near-isogenic lines of cultivars used in Brazil ('Ailsa Craig' WT vs. 'Ailsa Craig' *Tm-2²*), Nagai *et al.* (2019) found that five out of 10 plants with *Tm-2²* inoculated with ToMMV were positive in ELISA tests, but their absorbance values were much lower compared to samples of WT plants. Only one of the *Tm-2²* plants showed symptoms (mosaic and leaf deformation), whereas all WT plants were symptomatic. In addition, five (out of five) *Tm-2²*-plants inoculated with a ToMV-isolate also presented positive results for ELISA and one out of the five plants inoculated showed symptoms. Reduced plant size was observed for WT compared to *Tm-2²* plants. In conclusion, resistance breaking was not observed in the cultivar 'Ailsa Craig' *Tm-2²* (A. Nagai, pers. comm., 2021-11).
In relation to the severity of symptoms, one commercial tomato cultivar showed more severe symptoms after inoculation with a Brazilian ToMMV isolate than with a ToMV isolate but four other cultivars showed similar symptoms and one cultivar showed less severe symptoms; one cultivar was considered partially resistant against ToMV and symptomatic plants had similar symptoms as ToMMV-inoculated plants (Nagai *et al.*, 2019).
- Sui *et al.* (2016, 2017a, 2017b, 2018) and Chanda *et al.* (2021) reported on experiments with three tomato cultivars used for breeding resistance to TMV and ToMV (B, E, I – Brioso, Endeavour and Idolini – Chanda *et al.*, 2021). Sui *et al.* (2018), for ToMMV isolate MX5, report that some tomato plants of cultivar B (Brioso – with *Tm-2²* gene) kept in a greenhouse at 25-30°C were infected, while no plant of cultivar E (Endeavour – also *Tm-2²*) was infected, and cultivar I (Idolini – presence of *Tm-2²* not confirmed) was susceptible. The *Tm-2²* gene was confirmed to be present in both infected and non-infected plants of cultivar B (Sui *et al.*, 2017b). A temperature test observed an increasing number of ToMMV-inoculated cv. B plants infected as temperature increased from 25°C to 30°C or 35°C (Sui *et al.*, 2017b). The EWG noted that a temperature dependent reaction of plants carrying *Tm-2²* is known for ToMV (Pilowsky, 1981).
In one of the experiments, a delay in symptom expression was observed for plants of cultivar B with resistance breaking by ToMMV (necrotic lesions on systemic leaves nearly six weeks post-inoculation, while typical symptom expression could be visible in two weeks post-inoculation on susceptible cultivar B plants). All virus-infected susceptible plants (without any *Tm*-gene) were seriously stunted with aborted flowers and no fruit production, resulting in a total crop loss. In another experiment on 148 cultivar B plants, 15 plants (i.e. 10% of plants) were susceptible to ToMMV (Sui *et al.*, 2018).
Chanda *et al.* (2021) compared the resistance of the same three cultivars to ToBRFV, ToMMV (isolate SC13-05) and ToMV. In their experiments, the following infections rates were observed:
* on Endeavour 5.9% for ToMV, 16.7% for ToMMV;
* on Brioso 5% for ToMV; 10% for ToMMV.
* on Idolini 35% for ToMV, 25% for ToMMV.
The infection percentages were not significantly different between ToMV and ToMMV. Chanda *et al.* (2021) were puzzled by the proportions of Endeavour and Brioso plants being infected by ToMV and ToMMV and hypothesized that it might be due to some degree of cross-pollination with pollen from non-*Tm-2²* genotype(s) introducing susceptibility in the F1 hybrid population. More research is needed.
- In Mauritius (further details in section 6), in February 2021, disease incidence of 10% was observed on the cultivar Eliseo (cultivar with *Tm-2²*, highly resistant to ToMV) in a shade house. February is normally the hottest month in Mauritius (mean of 26.7°C recorded in February 2021) and the region where the disease was found was also among the warmest places in Mauritius. Temperature inside the shade house could have been around 35°C. Later during the year, ToMMV symptoms were observed in two other shadehouses with 75-80% infection on tomato varieties Policarpo (with *Tm-2²*) and Tropical Rose (not highly resistant), across the island, and even during winter months and in cooler parts of the island, when temperatures in shade houses were around 25°C (A Gungoosingh-Bunwaree, pers. comm. 2022-02). The EWG notes that the first case of infestation of a *Tm-2²* cultivar (Eliseo) may be due to *Tm-2²* temperature-sensitive nature, while no information is available on the factors that may have led to high incidences in the second case (Policarpo).
- Plantum (Dutch association of seed companies, after consultation of its members; pers. comm. 2021-12) noted that "*Tm-1* is not working against ToMMV, *Tm-2²* is effective (based on phenotypic test carried out by a breeding/seed company). Cultivars carrying *Tm-2²* were always resistant against ToMMV in these

tests. The same company indicates that all their indeterminate tomato cultivars³ have *Tm-2*². The tomato cultivars for fruit processing do not necessarily carry the resistance as this crop is not vulnerable for tobamoviruses because there is limited plant management – e.g. no hand labour like pruning. If *Tm-2*² would not be effective or lead to resistance break-down, we should have seen it much more in commercial circumstances”.

One Plantum member company noted that high temperatures lead to a hypersensitive response, which is reversible, but does not lead to resistance break down under natural circumstances (Plantum, Dutch association of seed companies, after consultation of its members; pers. comm. 2021-12). The *Tm-2*² temperature sensitive nature is mentioned in the original publication by Hall (1980). This is in line with the extensive fundamental research on N gene mediated HR in the tobacco model system (Whitham *et al.*, 1996).

► **The EWG concluded that the *Tm-2*² gene seems to be quite effective against ToMMV, although in some experiments and in two shade houses in Mauritius, symptomatic plants of cultivars carrying the *Tm-2*² gene have been found. In experiments, ToMMV-infection of plants of genotypes carrying *Tm-2*², sometimes asymptotically, occurred at a significantly lower percentage than for cultivars without resistance genes (Nagai *et al.*, 2019; Chanda *et al.*, 2021). There is one unexplained report of a high infection rate on a *Tm-2*² cultivar in one shade house in Mauritius. The reason for this is uncertain and more research is needed. Infection of plants carrying the *Tm-2*² gene may be linked to factors such as high temperature, genetic background of the cultivar, or proximity with highly infected crops (Betti *et al.*, 1997). Tomato cultivars that do not have the *Tm-2*² gene are more susceptible to ToMMV than cultivars that do have the *Tm-2*² gene.**

The EWG noted that cultivars carrying the *Tm-2*² resistance gene are widely used in crops with a higher risk of tobamovirus transmission due to higher levels of hands-on management, i.e. especially for the production of fresh fruit for consumption in greenhouse or in the field. Tomato cultivars for processing are less at risk of tobamoviruses because of limited hands-on management, and resistant cultivars are normally not used (K. Ling, pers. comm., 2022-03). The EWG noted that this is likely the case in a large part of the EPPO region with intensive commercial production of tomato (at least in the EU), although information is not available for all EPPO countries.

Furthermore, seeds may become contaminated by the virus during their production, and a proportion of seedlings grown from these seeds may then become infected (Betti *et al.*, 1997, as well as text above). Based on information available for other tobamoviruses, it is assumed that ToMMV is seedborne (at a low rate – see section 2.3). Seedborne infection is less likely in cultivars that carry *Tm-2*² resistance.

Pepper (*Capsicum* spp.)

For *Capsicum*, *L* genes/alleles⁴ providing resistance against tobamovirus infections have been introduced in pepper (*Capsicum* spp.) cultivars. Four *L* genes/alleles (*L*¹, *L*², *L*³, and *L*⁴) are known to provide increasing resistance to tobamoviruses, of which *L*² is not used in commercial varieties because it is rapidly overcome (ISF, 2012). In addition to these classical *L* genes/alleles, another gene/allele, *L*^{1a}, was more recently identified (Tomita *et al.*, 2011).

No reference or information was found on the resistance to ToMMV in relation to the *L* resistance genes/alleles in the literature. In resistance tests, Li *et al.* (2020) found different degrees of susceptibility/resistance amongst 10 *Capsicum* varieties: one variety (wild little pepper) was immune to infection by ToMMV, while others presented different grades of resistance to infection and disease severity. However, it was not possible to obtain information on whether these *Capsicum* varieties were harbouring *L* resistance genes/alleles.

³ Determinate tomatoes are cultivars that grow to a fixed mature size and ripen all their fruit in a short period (usually about two weeks). Indeterminate tomato cultivars are plants that continue to grow and produce tomato fruit throughout the growing season (<https://www.thespruce.com/indeterminate-tomato-variety-1403423>). The EWG noted that indeterminate tomato cultivars are usually grown in glasshouses, and determinate tomato cultivars usually in the field.

⁴ It is not known for certain whether differences in resistance are caused by allelic variants of the same gene, by presence/absence of different genes or by variations in different homologous genes. In this pest risk analysis, the different variants existing in pepper will be referred to as *L* resistance genes/alleles.

Plantum indicated that some parental *Capsicum* lines carrying *L1* and *L3* showed resistance in a small-scale experiment. *L4* was not tested but it is very likely that *L4* also confers resistance (Plantum, pers. comm., 2022-01).

Circumstantial evidence: so far, no outbreaks of ToMMV in *Capsicum* have been reported in the EPPO region; preliminary data from the Dutch seed collection suggest that ToMMV has been present before it was described in 2013 (see 6. Distribution), but no evidence in crops has so far been detected.

► **Limited evidence indicates that *Capsicum* cultivars carrying *L1* and *L3* may be resistant to ToMMV. *L4* may also confer resistance. Research is needed to confirm the efficacy of *L1*, *L3* and *L4* against ToMMV.**

According to EPPO (2020b citing a personal communication), mainly *L* resistant *Capsicum* are grown commercially in the EU and Israel. However, the EWG did not have data confirming that this is the case throughout the EU, and the EPPO region.

It is noted that, both for tomato and *Capsicum*, not all varieties used for breeding purposes to develop new cultivars may carry resistance genes.

No information on resistance to tobamoviruses was sought for other hosts.

2.8 Detection and identification methods

Detection

Generic PCR tests for tobamoviruses may be used for screening but also detect other tobamoviruses, such as the following end point RT-PCR or nested RT-PCR: Dovas *et al.*, 2004, Heinze *et al.*, 2006, Letschert *et al.*, 2002, Levitzky *et al.*, 2019, Li *et al.*, 2018, Luria *et al.*, 2017.

Specific molecular tests described in the identification section thereafter may also be used for the detection of ToMMV.

Li *et al.* (2021a) published three serological/enzyme-linked immunosorbent assays (ACP-ELISA⁵; dot-ELISA; tissue print-ELISA) using ToMMV-specific monoclonal antibodies for the detection of ToMMV in plant samples which were shown to not cross-react with ToMV, TMV, CGMMV and CMV. The detection limit of the dot-ELISA test is reported as being 26 times higher than that of end-point RT-PCR developed within their study, which amplifying *RdRp* fragment of ToMMV genome.

Previous to the development of these assays, no antibody for the detection of ToMMV was available (Sui *et al.*, 2017a; Zhan *et al.*, 2018), and polyclonal antisera raised against one tobamovirus showed cross-reactivity with one or more of the other tobamoviruses, including ToMV, TMV and ToBRFV (ISF 2019a&b, Sui *et al.*, 2017a & 2018; Webster *et al.*, 2014; Turina *et al.*, 2016; Zhan *et al.*, 2018). Cross-reactions with ToMV are reported in the literature to have caused misidentification because the two viruses share a high genetic similarity (see sections 1 & 7; Ambrós *et al.*, 2017; Nagai *et al.*, 2018). ELISA assays for the detection of tobamoviruses in *Capsicum* and tomato seeds are provided in ISF (2019a & b).

In general, analytical sensitivity of a bioassay is known to be lower than for ELISA and molecular tests. However, this method may be used for detection from symptomatic material, in which high virus concentrations are expected.

The ISHI-Veg local lesion bioassay for ToBRFV and ToMMV is not accepted as a valid test in New Zealand (NZ Ministry for Primary Industries, 2021).

In Australia, an end-point RT-PCR test has been validated for the detection of ToMMV (Levitzky *et al.*, 2019), because the same primers can be used for the detection of both ToBRFV and ToMMV on *Capsicum* and tomato seeds. PCR-amplified products from positive test results can be sequenced to confirm detection (Australian Government, 2019).

⁵ antigen-coated-plate enzyme-linked immunosorbent assay

The EWG noted that, although many EPPO countries (e.g. in the EU) conduct surveys as part of preventive measures against ToBRFV, these surveys mostly use ToBRFV-specific PCR methods, which will not detect ToMMV. In addition, serological tests currently in use for tobamoviruses do not differentiate ToMMV from ToMV or other tobamoviruses, and further tests are needed for a positive identification.

Identification

A multiplex species-specific PCR was developed for ToMMV, TMV and ToMV (Sui *et al.*, 2017a). Ambrós *et al.* (2017) developed a one-step endpoint RT-PCR and a northern hybridization assay with digoxigenin (DIG)-labelled RNA probes that can detect ToMMV and have been shown not to cross-react with ToMV and TMV. A one-step real-time RT-PCR assay was developed and validated for the simultaneous detection and identification of ToMMV and ToBRFV (Tiberini *et al.*, 2022). A ToMMV -specific RT-qPCR test was developed and validated by Fowkes *et al.* (2022).

Finally, endpoint RT-PCRs for ToMMV that do not cross-react with ToMV and TMV were developed by Turina *et al.* (2016) and by Zhan *et al.* (2018). However, validation data are missing and specificity was evaluated only with ToMV and TMV.

An Euphresco⁶ project on “Validation of molecular diagnostic methods for the detection and identification of tomato mottle mosaic virus” has been proposed in the 2022 call for topics.

Sequencing may be performed to identify ToMMV after amplification by generic tobamoviruses primers (see Generic RT-PCR tests in section 2.8). Complete nucleotide sequences have been determined for many isolates, such as: from Brazil (Nagai *et al.*, 2018), New York (Padmanabhan *et al.*, 2015), California and South Carolina (Sui *et al.*, 2017a), Mexico (Li *et al.*, 2013), Florida (Fillmer *et al.*, 2015), China (*Capsicum* isolates from Yunnan & Tibet – Li *et al.*, 2017; tomato isolates from Hainan – Zhan *et al.*, 2018, from Liaoning - Tu *et al.*, 2021) and Spain (Ambrós *et al.*, 2017).

Genome sequences of ToMMV are available in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>), as well as sequences originally allocated to ToMV and that are mentioned to be ToMMV (see Section 1 and Annex 1). Isolates that are in fact ToMMV are still indicated as being ToMV in Genbank, which may cause confusion and misidentification (as in Alavi and Massumi, 2014).

High throughput sequencing (HTS) technologies may be used for obtaining complete or almost complete genome sequences, which analysis can be used for identification of a virus isolate. HTS is used in some EPPO countries for example for routine testing during monitoring for the presence of viruses, and could detect and identify ToMMV. This method was used in studies that detected ToMMV on eggplant (Chai *et al.*, 2018 & 2019), *Cicer arietinum* (Pirovano *et al.*, 2015), tomato seeds (Fowkes *et al.*, 2022) and waste water (Furtak *et al.*, 2016) (see sections 6 and 7), but confirmation by another method is lacking in some of these cases.

3. Is the pest a vector?

Yes No

4. Is a vector needed for pest entry or spread?

Yes No

Tobamoviruses have no known natural vectors (Adams *et al.*, 2016). Bumblebees have been shown to be able to mechanically transmit ToBRFV from infected plants to healthy plants during pollination (Levitzy *et al.*, 2019). The EWG did not know of such studies for ToMMV.

5. Regulatory status of the pest

In the EPPO region:

- ToMMV is not listed as a quarantine pest by any EPPO country according to EPPO Global Database (EPPO, 2022). It was added to the EPPO Alert List in 2020 (EPPO, 2020a).

⁶ network for phytosanitary research coordination

- Turkey has notified to the WTO in 2021 emergency measures for several viruses, including ToMMV⁷, in relation to seeds of tomato and *Capsicum annuum* (WTO, 2021 G/SPS/N/TUR/119).
- Israel regulates tobamoviruses for the import of tomato and pepper seeds (MARD-PPIS, 2009). All imported tomato and pepper seeds undergo a general presence test for tobamoviruses, including ToMMV, upon arrival. In the revised phytosanitary regulations of Israel (in preparation), ToMMV is regulated under the requirements for tomato and pepper seeds (T. Levi, NPPO of Israel, 2021-12).

Information about the regulatory status of ToMMV elsewhere in the world was sought. The information consulted was not exhaustive and ToMMV may be regulated in more countries. From a search performed up to 2022-02-26:

- ToMMV is a regulated pest for Taiwan⁸ (WTO G/SPS/N/TPKM/568, 2021).
- ToMMV is a quarantine pest in Japan and emergency measures are in place (Zenshoku-kyo.or.jp, 2021a, b, c).
- Emergency measures targeting ToMMV have been introduced for seeds of tomato and *Capsicum* in Australia⁹ (Australian Government, 2019) and New Zealand¹⁰ (NZ Ministry for Primary Industries, 2021). In addition to the known hosts *C. annuum* and *C. frutescens*, the Australian measures also apply to *C. chinense*, and the New Zealand measures also to *C. baccatum*, *C. cardenasii*, *C. chinense*, *C. eximium*, *C. microcarpum*, *C. pendulum* and *C. pubescens*. See discussion on *Capsicum* in section 7.

In South Africa, the assessments of ToMMV and six pospiviroids are still pending (the tomato and *Capsicum* seed import requirements were amended in March 2019 to reflect measures for ToBRFV) (Sansor, 2020).

6. Distribution

ToMMV was first characterized in 2013 from samples collected in 2009 from infected tomatoes in Mexico (Li *et al.*, 2013). The earliest known collection of an isolate of ToMMV is 1992 in Sao Paulo State, Brazil (KT222999 originally identified as ToMV; Nagai *et al.*, 2018). Findings in the Dutch collection of tomato and *Capsicum* seeds (preliminary data 2022-03-10) also date back before the description of ToMMV as a separate species (see Table 1). To date, the virus has been detected by molecular methods in 11 countries in Asia, Europe, North America and South America (Table 1).

The distribution of ToMMV is uncertain. Sui *et al.* (2017a) and Li *et al.* (2020) note that it is likely to be more widely distributed than currently known. In particular, it cannot be excluded that some records of ToMV may relate to ToMMV. The finding in seed collections in the Netherlands (Table 1) and unconfirmed records (subsection at the end of the present section) also support the hypothesis of a wider distribution. To date, relatively few isolates have been found and sequenced (Sui *et al.*, 2017a citing Li *et al.* 2014, Turina *et al.* 2016).

In the EPPO region, there are no routine testings for ToMMV and it may have been overlooked. For seeds, it has been mostly found recently in relation to scrutiny for tobamoviruses. In production, it may have been thought to be ToMV, especially if ELISA tests were used (see also sections 1 and 2.7). ToMV outbreaks would not be reported.

Table 1. Distribution of ToMMV, including findings for which pest status in the country is not known

Continent	Country	Comments	References
EPPO region	Czech Republic	Pest status: present, few occurrences. See note below table.	EPPO, 2022; NPPO of the Czech Republic (J.

⁷ As of 15 September 2021, for seeds of tomato and *Capsicum annuum*, an additional declaration is requested on the phytosanitary certificate that the seeds originate from a country where ToMMV is not known to occur, or, if they originate from areas where the presence of ToMMV is known: the seeds originate in a place of production known to be free from the virus, and the seeds should have been tested by the exporting country by a RT-PCR method and found free. These requirements apply to ToMMV and several other viruses.

⁸ tomato and capsicum seeds should be tested and found free. Note: mentions countries where ToMMV is known to occur (Table 1 in section), as well as Vietnam (unconfirmed record in section 6).

⁹ tomato and capsicum seeds tested by PCR and found free

¹⁰ additional declaration on the PC that the seeds originate from a pest free area or pest free place of production for ToMMV, or have been tested and found free

Continent	Country	Comments	References
			Bustová, pers. comm., 2022-01)
	Israel	Absent. One finding in 2014 in a tomato glasshouse of a seed producer in Northern Israel (Turina <i>et al.</i> , 2016), not found since. See note below table.	EPPO, 2022; NPPO of Israel, 2021 (T. Levi, pers. comm., 2021-12)
	Netherlands	Found in the collection of tomato and <i>Capsicum</i> seeds of the Centre for Genetic Resources of the Netherlands. See note below table.	R. Lievers, Center for Genetic Resources of the Netherlands (pers. comm., 2022-02)
	Spain	Absent. One detection in 2015 on tomato in a research glasshouse near Valencia (Ambrós <i>et al.</i> , 2017). See note below table.	EPPO, 2022, NPPO of Spain, 2021 (B. Martínez Martínez, pers. comm., 2021-12)
South America	Brazil Minas Gerais Sao Paulo	At least since 1992.	Marubayashi <i>et al.</i> , 2017 Nagai <i>et al.</i> , 2018
North America	Mexico	Found in a greenhouse tomato sample (MX5) together with tomato necrotic stunt virus (potyvirus, Li <i>et al.</i> , 2014).	Li <i>et al.</i> , 2013
	USA (California, Florida, New York, South Carolina)	See note below table.	EPPO, 2022; Sui <i>et al.</i> 2017a (CA, SC); Webster <i>et al.</i> , 2014, Fillmer <i>et al.</i> 2015 (FL), Padmanabhan <i>et al.</i> , 2015 (NY)
Asia	China (Gansu, Hainan, Hunan, Liaoning, Neimenggu [Inner Mongolia], Shaanxi, Xizhang [Tibet], Yunnan, Shandong, Henan)	Present. See also interceptions further down.	Che <i>et al.</i> , 2018; Chen <i>et al.</i> , 2018; Chai <i>et al.</i> , 2019; Dong <i>et al.</i> , 2020 ; Li <i>et al.</i> , 2014, 2017, 2020, 2021b
	Iran	Detected, no details available. Several sequences deposited in GenBank as ToMV correspond to ToMMV. See section 1 / Annex 1 for details.	Li <i>et al.</i> , 2017, 2020; Turina <i>et al.</i> , 2016,
	India	Detected, no details available. See note below table.	ICAR-IIHR, 2017 & 2018
Africa	Mauritius	Few findings in 2021. See note below table.	Maudarbaccus <i>et al.</i> , 2021

Additional notes for some records in Table 1

Czech Republic. Surveys and tests in the Czech Republic have targeted tomato and *Capsicum* plants and seeds.

- In the field, ToMMV was detected in September 2020 on growing plants in three asymptomatic seed crops in Southern Moravia (two tomato crops of the varieties Mini and Datlo, and one *Capsicum annuum* crop). In 2020 seeds harvested from the three asymptomatic seed crops tested negative for ToMMV. Further tests were performed at the end of 2020 on the three lots of seeds harvested from the variety Mini: two were positive for ToMMV, the third was negative. In 2021, tests were again performed on seeds of the tomato variety Mini (harvested and ToMMV-positive in 2020) and the seed lot was again positive for ToMMV. Finally, a sample taken from a lot of tomato seed of the variety Datlo (seeds left from the 2020 sowing, from which one of the seed crops tested positive for ToMMV in 2020) was also retested and found positive (NPPO of the Czech Republic; J. Bustová, pers. comm., 2022-01).
- Other findings of ToMMV have been made on seeds of various origins (see section 8.1.1).
- 31 tomato samples from the Czech gene bank for testing on ToBRFV were tested for ToMMV, and were all negative for ToMMV (A. Přikrylová, pers. comm., 2022-03).

Israel. ToMMV was detected in 2014 in a tomato glasshouse of a seed company in Northern Israel (Turina *et al.*, 2016). The tomato plants were expected to be infected by TMV or ToMV as it concerned virus symptoms in plants without tobamovirus resistance genes (*Tm-1*, *Tm-2*, *Tm-2²*). The tomato crop was destroyed, and the entire greenhouse was cleaned and disinfected. In the subsequent tomato crops, only plants with tobamovirus resistance genes were used in the particular greenhouse and the virus has never appeared again. The origin of the virus remained unknown (B. Geraats, co-author of Turina *et al.*, 2016, pers. comm., 2021-12). ToMMV was not found in >1,000 ELISA tobamovirus-positive samples collected from most of the commercial tomato growers across the country, in November 2014–November 2016 (Luria *et al.*, 2017). The NPPO of Israel (T. Levi, pers. comm., 2021-12) noted that ToMMV is considered as a virus that is not present in Israel. There is not a specific official survey for the virus, but when suspicious plants and fruits are brought to the laboratory, they are also tested for presence of ToMMV, and no findings have been recorded so far. Similarly, no findings on the presence of the ToMMV have been received since the 2014 report.

Netherlands. Systematic testing of the tomato and pepper seed collections (1548 and 1331 accessions, respectively) of the Centre for Genetic Resources of the Netherlands (CGN) by real-time PCR, showed that 92 out of 102 tomato seed bulks and 40 out of 99 pepper seed bulks (each bulk containing on average approximately 15 (tomato) or 13 (pepper) accessions) tested positive for ToMMV. Accessions were grouped per production year and production location. A bulk only contained seeds from the same year and the same production location (with a few exceptions). When bulking, seeds were handled in a manner preventing cross-contamination. Most positive bulks during systematic testing were from the Netherlands, but some were from other EPPo countries. Test results (low Ct-values) suggest that at least seeds of tomato produced in 2007 were infested during that year of production. For other accessions investigations are going on to find out in which year(s) the seeds may have become infested (R. Lievers, CGN, pers. comm., 2022-02-10). RNA-extracts from 18 of the seeds bulks that had tested positive for ToMMV by PCR were also analysed by high throughput sequencing and ToMMV could be identified in each of these extracts including seeds that had been produced in three different EU member states (preliminary results, M. Botermans & D.J. van der Gaag, NVWA, the Netherlands, 2022-05).

ToMMV was also identified in one case in tomato seeds “harvested from plants which were part of a selection programme of candidate varieties grown in The Netherlands” (Fowkes *et al.*, 2022).

In commercial fruit crops, ToMMV appears, however, absent in the Netherlands. As part of the annual survey programme, approximately 125 tomato fruit and 125 *Capsicum* fruit greenhouses are inspected every year in the Netherlands. In case of suspicious symptoms, samples are taken and tested for several viruses including ToMMV. So far, ToMMV has not been detected. In addition, as part of the official measures against ToBRFV, illumina sequencing was used on 86 samples of tomato fruit crops from approximately 59 greenhouses that tested positive for ToBRFV. ToMMV was not detected in these samples (situation on 7 March 2022; pers. comm. M. Botermans & D.J. van der Gaag, NVWA).

Spain. ToMMV was detected in 2015 on tomato in a research glasshouse near Valencia; the origin of the infestation was unknown (Ambrós *et al.*, 2017). The competent authority of the Valencia region informed the NPPO of Spain that there is no evidence of the presence of ToMMV in the Valencia region, despite the fact that extensive monitoring is conducted on tomato because of the preventive measures for ToBRFV. Therefore,

the status of ToMMV in Valencia region is 'absent' (NPPO of Spain; B. Martínez Martínez, pers. comm. 2021-12).

USA. The record for California is based on Sui *et al.* (2017a - tomato samples collected from a greenhouse in 2016). However, the grower took measures to clean out the greenhouse, no more additional outbreak was reported for ToMMV from that greenhouse since 2016 (K-S. Ling, pers. comm., 2022-02). Following an earlier report, Chitambar (2015) mentioned that ToMMV was not known to be established in California because measures were applied following the finding of the virus by a seed company in a farm site in San Joaquin County.

The record for South Carolina is based on Sui *et al.* (2017a) and in New York based on Padmanabhan *et al.* (2015 – experimental tomato plants in greenhouse). In both cases, measures were taken following these findings, and there has been no additional ToMMV outbreaks reported from those states (K.-S. Ling, pers. comm., 2022-02).

In Florida, ToMMV was identified in samples collected in 2010 in a commercial tomato field and in 2012 in a commercial greenhouse (Webster *et al.*, 2014, Fillmer *et al.* 2015), but no outbreak appears to have been reported since (S. Adkins, pers. comm., 2022-03). See also interceptions further down.

India. ToMMV was identified from infected tomato based on electron microscopy, host range and ELISA testing. In addition, the complete genome nucleotide sequence "had the highest nucleotide similarity of 92.2 to 92.3% with ToMMV isolates from Brazil, China, Spain and USA, 61.6 to 83.8% with Tobamoviruses of Solanaceous hosts and 42.2 to 57.7% with other tobamoviruses"; no details are provided on location or conditions of the finding (ICAR-IIHR 2017, page 60, in a section on research achievements). The 2017-2018 annual report (ICAR-IIHR, 2018) lists the following paper presented at the "International Symposium on Horticulture Priorities & Emerging Trends, September 5-8, 2017, IISc Campus, Bengaluru, India»: *Suveditha S, Hema Chandra Reddy P, Salil Jalali and Krishna Reddy M - Identification and diagnosis of Tomato mottle mosaic virus in Tomato*. The virus is not mentioned in subsequent reports. No further information was found. See also interceptions further down.

Mauritius: In February 2021, ToMMV was observed in an insect-proof shade house on about 10% of plants. The origin of the virus is suspected to be from seeds since it was first observed in very few plants but later spread due to cultural practices. Later ToMMV symptoms were observed in two other shadehouses with 75-80% infection. Again, the source of the virus was suspected to be infected seeds since these shade houses were far from each other (Maudarbaccus *et al.*, 2021; A. Gungoosingh-Bunwaree, Food and Agricultural Research and Extension Institute, Mauritius; pers. comm. 2022-02; details on cultivars are in section 2.7). An awareness campaign was launched and surveillance intensified in tomato crops (Maudarbaccus *et al.*, 2021). The measures applied have prevented the spread of the virus, which, by August 2021, had not been found in other tomato or solanaceous crops on the island; surveillance continued (MauriceActu, 2021). The virus has not been found either on Rodrigues Island as of March 2021 (Rodrigues regional assembly, 2021). Tomato is grown all-year round in Mauritius. So far the disease has not been observed in open field (A. Gungoosingh-Bunwaree, Food and Agricultural Research and Extension Institute, Mauritius; pers. comm. 2022-02).

Information received from other EPPO countries

In some EPPO countries, surveys have been conducted and have not detected ToMMV to date. For example:

- In Slovenia, 86 official samples (each sample represented one to five plants) of tomato (54) and pepper (32) grown in the period 2017-2021 were tested by HTS. ToMMV has not been detected in any of tested samples. In addition, 293 tomato plants sampled for research purpose in 2019-2020 were tested by HTS and ToMMV was not found (D. Kutnjak & N. Mehle, pers. comm., 2022-02).
- In Finland, 108 tomato and 21 pepper leaf samples were collected as part of official inspections of vegetable production in 2019. Each sample contained leaves from 20 individual plants. These samples were tested for viruses by HTS (as a part of a research project). ToMMV was not detected in any of the samples (J. Santala, pers. comm., 2022-02).
- In Italy, 52 official samples of tomato grown in the period 2020-2021 were tested by real time RT-PCR (Tiberini *et al.*, 2022). ToMMV has not been detected in any of tested samples. In addition, 124 official samples of seeds (65 tomato and 59 pepper) originating from Italy were sampled and tested in the period from 2020 to February 2022 by real time RT-PCR and ToMMV was not found (A. Tiberini, pers. comm., 2022-03).

Unconfirmed records

- **Vietnam and Myanmar.** Taiwan's regulation on ToMMV (WTO G_SPS_N_TPKM_568, 2021) cites Vietnam in addition to all countries in Table 1. Following the interception in Japan of ToMMV in *Capsicum* seeds imported from Taiwan, the Taiwanese authorities communicated that the *Capsicum* seeds originated from 'Vietnam and Myanmar' (Zenshoku-kyo.or.jp, 2021b & c). A survey for the identification of viruses and viroids infecting tomato and *Capsicum* plants in Vietnam by metatranscriptomics did not identify ToMMV (Choi *et al.*, 2020). Finally, ToMMV has been intercepted at several occasions in seeds from Vietnam (details in section 8.1.1).
- **Italy.** ToMMV was detected by HTS on *Cicer arietinum* (Pirovano *et al.*, 2015). This host record is considered uncertain (see section 7), and therefore the presence in Italy as well. ToMMV has not been reported on other plants. In addition, ToMMV was recently reported using a proteomics approach in Sicilia on tomatoes bought on a market (Mammadova *et al.*, 2021). However, this is not considered to be a finding of ToMMV because there was 100% identity to both ToMV and ToMMV capsid protein.
- **Pakistan.** A study on the presence of viruses in sewage water detected ToMMV MX5 isolate in samples from Islamabad by HTS (Furtak *et al.*, 2016¹¹).
- **Others linked to interceptions.** ToMMV has been found in several countries on tomato and *Capsicum* seeds from various countries of origin (see details in section 8.1.1, Table 4). However, the true origin of the seeds is not known in all cases (as shown in Table 3). Several countries of origin of infested consignments are already detailed above, as confirmed records (USA, China, India) or uncertain records (Italy, Vietnam). For the following countries of origin, no information was found about the presence of ToMMV:
EPPO Slovakia, Poland, the Netherlands (other than in collection), Spain.
non-EPPO Chile, Guatemala, Indonesia, Japan, Thailand.

7. Host plants and their distribution in the PRA area

Known natural hosts

Most natural infections of ToMMV relate to tomato (*Solanum lycopersicum*) and *Capsicum* (*C. annum*, *C. frutescens*). However, the virus has also been detected in the field on tobacco (*Nicotinia tabacum*, Solanaceae), kidney bean (*Phaseolus vulgaris*), pea (*Pisum sativum*) (both Leguminosae), and on the weed nettle-leaved goosefoot (*Chenopodium murale*, Chenopodiaceae) (Table 2).

ToMMV may have more hosts. The related ToMV is known to infect more plant species and some isolates previously identified as ToMV turned out to be ToMMV.

Nicotiana tabacum (Dong *et al.*, 2020). In 2017-2018, 303 tobacco symptomatic samples were collected from five tobacco production areas in Hunan province in China. ToMMV was detected by RT-PCR (TMV was the most dominant virus, followed by CMV and PVY). 255 contigs out of 4057 contigs were highly homologous with ToMMV, covering samples from all five tobacco production areas. The article does not provide a specific description of the positive samples, and of the number of samples found infested by ToMMV. It is mentioned that co-infections by viruses reached 71.29% and single infection were only 28.71%, but it is not mentioned if ToMMV was present in co-infections or single infections. Similar to eggplant, no conclusion can be made about the pathogenicity of ToMMV on tobacco. Nevertheless *N. tabacum* was shown to be a symptomatic host in many inoculation studies (Ambrós *et al.*, 2017 - alterations at phenotypic level; Li *et al.*, 2017, 2020 (positive RT-PCR, with some seed to seedling transmission), Sui *et al.*, 2017a systemic; Turina *et al.*, 2016; Webster *et al.*, 2014) and is considered as a host in this PRA.

It is proposed to consider for this PRA that all *Capsicum* spp. (including *C. annum*, *C. chinense* and *C. frutescens*) are hosts because the three species are closely related; and hybrids are commonly bred and distributed globally. In addition, seeds of the three *Capsicum* species are similar in appearance and thus difficult to distinguish. Seed traders usually only identify seeds using the name '*Capsicum*'.

¹¹ Note that the conclusion text reads 'tomato mottle virus', but that the two tables in the article list tomato mottle mosaic virus MX5, and MX5 is the strain mentioned amongst others in Turina *et al.* (2016).

The record on *Ph. vulgaris* relates to ‘kidney beans’, but all *Ph. vulgaris* are covered in this PRA (incl. green beans). The term “common beans” is used in this PRA to refer to *Ph. vulgaris* only (and not other *Phaseolus* sp. or *Vigna* sp.).

Capsicum spp., *S. lycopersicum*, *P. sativum* and *Ph. vulgaris* are widely grown in the EPPO region, while *N. tabacum* is cultivated in part of the region (see section 9).

Experimental hosts

In laboratory experiments, ToMMV was mechanically transmitted to a wide range of plants in the families Solanaceae, Amaranthaceae, Asteraceae, Brassicaceae, Cucurbitaceae, Leguminosae and Verbenaceae (Table 2)

A recent study on host range provided comparative analyses on the same host species among three tomato-infecting tobamoviruses (ToBRFV, ToMMV and ToMV). Although there are many similarities, there are some unique host plants that may be used to differentiate these tobamoviruses biologically. For example: *Emilia sonchifolia* and *Nicotiana debneyi* are hosts for ToBRFV, but not for ToMMV and ToMV; *Datura stramonium* and *Nicotiana tabacum* ‘Xanthi nc’ are hosts for ToBRFV and ToMMV, but not for ToMV. On the other hand, *Verbena officinalis* var. *halei* is not a host for ToBRFV, but are the hosts for ToMMV and ToMV (Chanda *et al.*, 2021).

Certain species were found to be experimental hosts in some studies but not others, and this is indicated in Table 2 for those species. Finally, in experiments, no symptoms and no systemic infection were found on the following plants: *Citrullus lanatus*, *Cucumis melo*, *Cucumis metulifer* (all Cucurbitaceae), *Brassica rapa*, *Lactuca sativa*, *Emilia sonchifolia* and *Catharanthus roseus* (Sui *et al.*, 2017a), *Luffa cylindrica*, *Momordica charantia*, *Vigna unguiculata* and *Ipomoea aquatica* (Li *et al.*, 2020).

Doubtful host records and records that need confirmation

There is a uncertainty on whether the following plant species are hosts:

- *Solanum melongena*. ToMMV was reported on eggplant in China, only in mixed infections with tobacco mild green mosaic virus (TMGMV) in plants collected from several farms; the viruses were identified using high throughput sequencing and screening using bioinformatic methods, followed by RT-PCR and pathogenicity tests (Chai *et al.*, 2018 & 2019). Evidence is lacking of whether ToMMV can infect eggplant on its own. The pathogenicity of ToMMV on eggplant is also not confirmed because pathogenicity tests were performed with both viruses together. *S. melongena* was not a host in inoculation studies in Sui *et al.* (2017a – only necrotic local lesions on leaves) and is mentioned as a non-host in Chanda *et al.* (2021), but it was an experimental host in Li *et al.* (2020: mottle & necrosis and later detection by PCR – see * note below Table 2). No infection were found in China during surveys in 13 provinces (Li *et al.*, 2020), but few eggplant samples (19) were collected during this study. No further information was obtained.
- *Cicer arietinum* (Leguminosae). ToMMV was detected by metagenomics (deep sequencing) in *C. arietinum* in Italy, in plants grown from seeds of an old cultivar in a collection in Puglia (Pirovano *et al.*, 2015). The seeds were of Italian origin (local, yearly propagated from the same area of production) (V. Pirovano, pers. comm. 2021-12). V. Pirovano (pers. comm.; 2021-10) notes that “the presence of a tobamovirus into old varieties of chickpea in Puglia is not surprising since this genus of plant viruses is known to be hosted by a wide range of plant species, including legumes. Moreover, all viral species are known to be transmitted mechanically and also through contaminations of the seed teguments (Broadbent, 1965).” No tests were done on the seeds from the same accession used to grow the plants that were found infested, but a project is planned to investigate viruses contaminating seed from chickpea and other old varieties of legumes; the origin of the virus or mode of transmission was not investigated, so it is not known if the plants were infested by another means (e.g. mechanical). From the above, the host status of *C. arietinum* needs confirmation: ToMMV was only detected once and its presence was not confirmed by any other method.

Table 2. Hosts of tomato mottle mosaic virus under field conditions (confirmed hosts) and in laboratory experiments (experimental hosts)

	Presence in PRA area	Comments	References for host status
CONFIRMED HOSTS			

	Presence in PRA area	Comments	References for host status
Solanaceae			
<i>Capsicum annuum</i>	Yes, widely grown	Found in China	var. <i>grossum</i> ; Li <i>et al.</i> , 2014, 2017
<i>Capsicum frutescens</i>	Yes, widely grown	Found in China	Li <i>et al.</i> , 2014, 2017
<i>Capsicum</i> spp.	Yes, widely grown	Three isolates from Brazil and Iran identified as ToMV but corresponding to ToMMV	Li <i>et al.</i> 2017, 2020
<i>Solanum lycopersicum</i>	Yes, widely grown	Found in all countries where ToMMV is known to be present	Li <i>et al.</i> 2017, 2020; Turina <i>et al.</i> , 2016, Fillmer <i>et al.</i> , 2015 etc.
<i>Nicotiana tabacum</i>	Yes, grown in part of the EPPO region	Found in China. Also Experimental host.	Field: Dong <i>et al.</i> , 2020. Experimental: Ambrós <i>et al.</i> , 2017 alterations at phenotypic level; Li <i>et al.</i> , 2017, 2020 (positive RT-PCR), Sui <i>et al.</i> , 2017a systemic; Turina <i>et al.</i> , 2016; Webster <i>et al.</i> , 2014
Leguminosae			
<i>Phaseolus vulgaris</i>	Yes, widely grown	Found in Iran: one plant in the field, cv. Red kidney. The isolate groups with Brazilian isolates now known to be ToMMV. Sequences identified as being ToMMV (Li <i>et al.</i> , 2017, 2020). Also experimental host in China (Li <i>et al.</i> , 2020): Symptomatic beans infected with ToMMV have symptoms of mottle and necrosis (Figure 4-D in article). ToMMV-specific detection method on pods and bean seeds can detect ToMMV (Li <i>et al.</i> 2020)	Correct identity mentioned in Li <i>et al.</i> 2017 & 2020 Isolate in Alavi & Massumi, 2014 Li <i>et al.</i> 2020 symptoms and positive RT-PCR
<i>Pisum sativum</i>	Yes, widely grown	Found in China: two symptomatic plants in the field	Zhang <i>et al.</i> , 2021
Chenopodiaceae			
<i>Chenopodium (=Chenopodium) murale</i>	Native. Yes, wild/weed	Found in Iran, isolate identified as ToMV (in Aghamohammadi <i>et al.</i> , 2013) but sequences in Genbank correspond to ToMMV (Li <i>et al.</i> , 2017, 2020).	Correct identity mentioned in Li <i>et al.</i> 2017 & 2020 Isolate from Aghamohammadi <i>et al.</i> , 2013

	Presence in PRA area	Comments	References for host status
		Aghamohammadi <i>et al.</i> (2013) sampled asymptomatic Chenopodiaceae weeds “within fields and field margins of various vegetable settings”	
EXPERIMENTAL HOSTS			
Solanaceae			
<i>Datura stramonium</i>	Native or naturalised. Yes, wild/weed	Symptomatic	Sui <i>et al.</i> , 2017a systemic [Not experimental host in Li <i>et al.</i> , 2020 asymptomatic and negative RT-PCR]
<i>Nicandra physaloides</i> *	Exotic. Yes, as ornamental	Symptomatic	Li <i>et al.</i> , 2017, 2020 positive RT-PCR
<i>Nicotiana benthamiana</i>	No, model organism	Symptomatic	Sui <i>et al.</i> , 2017a systemic, Webster <i>et al.</i> 2014, Zhan <i>et al.</i> , 2018, Li <i>et al.</i> , 2017, 2020 positive RT-PCR, Ambrós <i>et al.</i> , 2017 alterations at phenotypic level
<i>Nicotiana glutinosa</i> *	Yes, in tobacco hybrids, model organism	Symptomatic	Li <i>et al.</i> , 2020 positive RT-PCR [Not experimental host in Webster <i>et al.</i> , 2014: only necrotic local lesions]
<i>Nicotiana rustica</i>	Wild tobacco. In Spain, not found as crop, occasionally in the wild. No information sought for the rest of EPPO	Symptomatic	Sui <i>et al.</i> , 2017a systemic; Li <i>et al.</i> , 2017, 2020 positive RT PCR
<i>Petunia hybrid</i>	Yes, as ornamental	Symptomatic	Sui <i>et al.</i> , 2017a systemic; Li <i>et al.</i> , 2017, 2020 positive RT-PCR
<i>Physalis alkekengi</i> *	Native. Yes, wild, also as ornamental	Symptomatic	Li <i>et al.</i> , 2017, 2020 positive RT-PCR
<i>Physalis angulata</i>	Exotic. Yes, wild/weed?	Symptomatic	Sui <i>et al.</i> , 2017a systemic
<i>Physalis pubescens</i>	Exotic. Yes, wild/weed?	Symptomatic	Sui <i>et al.</i> , 2017a systemic
<i>Solanum nigrum</i>	Native. Yes, wild/weed.	Symptomatic	Sui <i>et al.</i> , 2017a systemic
<i>Solanum pimpinellifolium</i>	Currant tomato. Wild ancestor of cultivated tomato. Sold as garden plants (not as crop)	Symptomatic	Nagai, 2017, Nagai <i>et al.</i> , 2020 infected
<i>Solanum tuberosum</i> *	Potato. Yes, widely grown	Symptomatic	Li <i>et al.</i> , 2020 positive RT-PCR
Amaranthaceae			
<i>Chenopodium amaranticolor</i>		Symptomatic (AF4111922 from Brazil)	Originally published by Moreira <i>et al.</i> , 2003 as ToMV. Mentioned as

	Presence in PRA area	Comments	References for host status
			being ToMMV in Li <i>et al.</i> (2017, 2020) and Turina (2016).
<i>Chenopodium quinoa</i>	Quinoa. Yes, as crop (limited) (Bazile <i>et al.</i> , 2016)	Symptomatic	Ambrós <i>et al.</i> , 2017 alterations at phenotypic level Sui <i>et al.</i> , 2017a systemic [Not experimental host in Li <i>et al.</i> , 2020: no symptom and negative RT-PCR]
<i>Gomphrena globosa</i>	Exotic. Yes, as ornamental	Symptomatic	Sui <i>et al.</i> , 2017a systemic
Asteraceae			
<i>Glebionis coronaria</i>	Native. Yes, wild and ornamental	Asymptomatic	Sui <i>et al.</i> , 2017a systemic
Brassicaceae			
<i>Arabidopsis thaliana</i>	Native. Yes, wild/weed Prototypical experimental model system in plant biology	Symptomatic	Ambrós <i>et al.</i> , 2017 alterations at phenotypic level
<i>Brassica campestris</i> *	<i>B. rapa</i> subsp. <i>sylvestris</i> Yes, wild and crop	Symptomatic	Li <i>et al.</i> , 2017, 2020 positive RT-PCR
<i>Brassica chinensis</i> *	<i>Brassica rapa</i> subsp. <i>chinensis</i> . Yes, as crop	Symptomatic	Li <i>et al.</i> , 2017, 2020 positive RT-PCR
<i>Brassica oleracea</i> var. <i>italica</i> *	Broccoli. Yes, as crop	Asymptomatic	Li <i>et al.</i> , 2017, 2020 positive RT-PCR
<i>Brassica oleracea</i> var. <i>botrytis</i> *	Cauliflower. Yes, as crop	Asymptomatic	Li <i>et al.</i> , 2017, 2020 positive RT-PCR
<i>Brassica pekinensis</i> *	<i>Brassica rapa</i> subsp. <i>pekinensis</i> . Chinese cabbage. Yes as crop	Symptomatic	Li <i>et al.</i> , 2017, 2020 positive RT-PCR
<i>Raphanus sativus</i> *	Radish. Yes, as crop.	Symptomatic	Li <i>et al.</i> , 2017, 2020 positive RT-PCR
Cucurbitaceae			
<i>Cucumis sativus</i> *	Cucumber. Yes, as crop	Asymptomatic	Li <i>et al.</i> , 2020 positive RT-PCR
<i>Cucurbita moschata</i> *	Musky gourd. Yes, as crop	Asymptomatic	Li <i>et al.</i> , 2020 positive RT-PCR [Not experimental host in Sui <i>et al.</i> (2017a) no symptoms, nor detected by RT-PCR in non-inoculated leaves]
<i>Cucurbita pepo</i> *	Pumpkin. Yes, as crop	Symptomatic	Li <i>et al.</i> , 2020 positive RT-PCR
Verbenaceae			
<i>Verbena halei</i> (as <i>Verbena officinalis</i> var. <i>halei</i>)	Exotic (N.Am.) Yes, as ornamental and medicinal	Asymptomatic	Sui <i>et al.</i> , 2017a systemic

* Experimental hosts listed in Li *et al.* (2020) are considered uncertain because information is lacking on whether the positive RT-PCR tests were performed on new leaves (in which case the plant is a confirmed

experimental host) or on inoculated leaves (local hypersensitive reaction). This was not documented in the article, and it was not possible either to obtain further information.

8. Pathways for entry

ToMMV is known to be transported in plants and seeds of hosts, and soil transmission has been shown. Tobamoviruses are also transmitted by mechanical contact (section 2.1).

Hosts covered in the pathways

In principle, all hosts are covered in the pathways: tomato, *Capsicum*, tobacco, peas and common beans. However, there is little information regarding ToMMV in tobacco, peas and common beans. There are few records of natural infection on these species, and insufficient evidence regarding association with these plants and with crops (see section 7). There are many more records for tomato and *Capsicum*, and seeds and plants for planting of tomato and *Capsicum* were considered the most important pathways. Therefore, only these host plants were considered in detail in the pathway-analysis. The EWG noted that pathways related to tobacco, peas and common beans may need to be assessed if further evidence becomes available, and such pathways are listed further down.

The following pathways for entry of ToMMV were discussed in this PRA. Pathways in bold are described and evaluated in section 8.1 while the other pathways that are considered ‘unlikely pathways’ are described in section 8.2.

- **Seeds of tomato and *Capsicum* (Table 3 in this section)**
- **Plants for planting (excluding seeds and pollen) of tomato and *Capsicum* (Table 4 in this section)**
- **Fruit of tomato and *Capsicum* (Table 5 in this section)**
- **Used containers, tools, equipment and conveyance vehicles associated with host production and supply chain**
- Travellers (persons working in place of production of host plants)
- Natural spread, from countries where ToMMV occurs to EPPO countries where it does not occur
- Pollinating insects (from third countries) used in host fruit production
- Soil and growing media on its own
- Pollen of host plants
- Processed and dried fruits of hosts
- Soil or growing media attached to non-host plants

The following pathways may be of relevance for tobacco, peas and common beans, and their assessment may become relevant in the future if information is available: seeds; plants for planting (except seeds and pollen); *Pisum sativum* and *Phaseolus vulgaris* fruit and grain; tobacco plant parts and processed products of tobacco.

Experimental hosts

Potential pathways related to experimental hosts are not covered in this PRA as there is no evidence that these hosts are susceptible to natural infection. This relates to: seeds, plants for planting, cut flowers (e.g. *Petunia*), dried plant parts (e.g. branches of *Physalis alkekengi*, dried leaves of *Verbena halei*), fresh plant parts (e.g. broccoli, cauliflower, radish, *V. halei*), fruit of *Cucumis* and *Cucurbita* experimental hosts. In case of confirmation of natural infection of other experimental hosts, additional pathways may need to be considered to re-evaluate the risk of entry.

8.1 Pathways investigated in detail

Information on import prohibitions and phytosanitary measures is not provided for all countries in the PRA area.

8.1.1 Seeds of tomato and *Capsicum*

Because of the similarities with ToBRFV, the study of pathways is mostly based on that of ToBRFV, with added consideration for tobacco, pea and common bean.

Table 3. Seeds of tomato and *Capsicum*

Seeds of tomato and <i>Capsicum</i>					
Pathway	Seeds of tomato and <i>Capsicum</i>				
Coverage	Seeds of tomato and <i>Capsicum</i>				
Plants concerned	<p><i>S. lycopersicum</i>, <i>Capsicum</i> spp.</p> <p>ToMMV has been found associated only with seeds of tomato and <i>Capsicum</i> to date, but like other tobamoviruses, it may also be associated with seeds of other hosts.</p>				
Pathway prohibited in the PRA area?	No				
Pathway subject to a plant health inspection at import?	<p>Yes, at least in some EPPO countries.</p> <p>For example, in the EU, a phytosanitary certificate (PC) and import inspection are required for imported seeds of tomato and <i>Capsicum</i>.</p> <p>In the UK, all seed for planting from outside the EU require a PC. The following relevant seed dispatched from the EU requires a PC: <i>S. lycopersicum</i> and <i>Capsicum</i> spp.</p> <p>The EWG had no information on the requirements of non-EU EPPO countries.</p>				
Pest already intercepted?	Yes.				
	Seeds of	Origin/exporting country (number, if specified)	Intercepted by or details	Year (when specified)	reference
	tomato	China	Czech Republic		Dutch NPPO, 2020
	tomato	India (via the USA)	UK		Dutch NPPO, 2020
	tomato	Vietnam (ex Japan) (1) India (2)	UK	2020	UK PRA, 2021, unpublished
	tomato	unknown origin (1) India (3) China (1) Japan (1) Thailand (1) USA (1)	UK	2021 (up to June)	UK PRA, 2021, unpublished Some findings from Asia (incl. China and two countries not specified in the article) are detailed in Fowkes et al. (2022)
	<i>C. annuum</i>	China (1) Indonesia (2)	UK	2021	EPPO RS 2021/238
	tomato	Guatemala (1) Italy (1) Japan (1)	UK	2021	EPPO RS 2021/238

Pathway	Seeds of tomato and <i>Capsicum</i>				
		USA (1)			
	Seeds of	Origin/exporting country(number, if specified)	Intercepted by or details	Year (when specified)	reference
	<i>Capsicum</i>	Netherlands Spain	Australia		Australian Government, 2019
	tomato	USA	Australia		Australian Government, 2019
	tomato	China (in five samples; cvs: Amalia, Chiquita pot, Imola, Ruxandra, Sandybelle)	106 tomato and pepper official seed samples (RNA extracted from 62 tomato seed samples and from 44 pepper seed samples) from different origins (China, Italy, Brazil, India, Slovenia, Serbia) and previously analyzed for ToBRFV, were later tested for ToMMV.	2021	Tiberini <i>et al.</i> , 2022
	tomato	USA China (directly and also via Israel*)	Spain - tomato samples and 85 pepper samples from Chile, China, India, Israel, USA, Thailand and Turkey were analysed by RT-PCR	Between Nov. 2019-July 2021	Mut Bertome, 2021
	<i>Capsicum</i>	Vietnam or Myanmar (via Taiwan)	Japan		See section 6
	<i>Capsicum</i>	Chile (via the Netherlands**)	Turkey	2022-01	NPPO of the Netherlands
	<i>Capsicum</i>	Vietnam (via the Netherlands**)	Thailand	2022-02	NPPO of the Netherlands
	<p>* An investigation of the finding in seeds from Israel was conducted with the Israeli seed company and the findings showed that the seed originated from China (NPPO of Israel; T. Levi, 2021-12).</p> <p>** The seeds intercepted by Turkey from the Netherlands originated from Chile, and those intercepted by Thailand from the Netherlands originated from Vietnam (NPPO of the Netherlands).</p>				
Most likely stages that may be associated	ToMMV has been found associated with seeds of tomato and <i>Capsicum</i> , and it may be seed-borne (section 2).				
Important factors for association with the pathway	Usually, seeds of varieties intended for glasshouse fruit production (high-value varieties) are produced indoor, while open pollinated seeds (non-hybrids) of public varieties (publicly available varieties) or seeds of varieties intended for industry (lower value varieties) are produced outdoor (Lybeert, pers. comm., 2020 cited in EPPO, 2020b). Low value hybrid seeds are sometimes also produced outdoor (e.g. in the Gansu province, China or in Thailand) with several manipulations (Desulauze, pers. comm., 2020 cited in EPPO, 2020b). The likelihood of association may depend on the way the seed is produced (e.g. less handling with open pollinated seeds) but the EWG did not have enough information to make separate ratings for the likelihood of association for different kind of varieties (hybrids				

Pathway	Seeds of tomato and <i>Capsicum</i>																
	<p>versus non-hybrids and indoor versus outdoor production). In addition, almost all the imported seed is thought to be first generation (F1) hybrid¹² seed produced by cross-pollination (hybridisation) of parental lines (AGDAWR, 2018).</p> <p>The following factors may affect the association of the pest with the seeds. <u>These factors are fully developed in Annex 4.</u></p> <ol style="list-style-type: none"> 1- It is difficult to traceback the geographical origin of seed lots and therefore to be certain of its pest status. Individual consignments of hybrid seeds are often composed of mixed lots of seeds produced in several countries of origin, which increases the probability of association of the virus with the seed and the presence of infected seed in the individual seed lots derived from the bulked seed. 2- By growing plant lines in different places, the plant lines may be exposed to a higher likelihood to get infected 3- Combining seeds from different sources into a single consignment may also introduce infected seeds to healthy seed lots. 4- The seed production process involves many steps and manipulations which may be a source of mechanical transmission and spread of the disease. 5- The seed production process involves regular cleaning, heat and chemical treatments which may reduce the association of the virus with the seed. <p>See Annex 4 Point 5 in relation to treatments applied against ToMV in some countries during seed production.</p> <p>6- Existing field inspections for other pests and seed-lots testing for tobamoviruses prior to export reduce the likelihood of association.</p>																
Survival during transport and storage	<p>Survival of the virus in the seed coat: After extraction and treatment, seed lots may be stored for several years (AGDAWR, 2018) but this is unlikely to significantly affect the survival of the virus. Eighty-five percent of cucumber seeds inoculated with CGMMV were found infested after four years (Dombrovsky, pers. comm., 2019 cited in EPPO, 2020b). Survival would be more limited by the impact of storage on seed germination.</p>																
Trade	<p>Almost all the imported seed is thought to be first generation (F1) hybrid¹³ seed produced by cross-pollination (hybridisation) of parental lines (AGDAWR, 2018).</p> <p><u>Tomato, <i>Capsicum</i></u></p> <p>Trade data available in Eurostat (i.e. imports into EU countries) cover ‘vegetable seeds for sowing (excluding salad beet or beetroot ‘Beta vulgaris var. conditiva)’ (EU CN code 12099180). There are no specific data for tomato and <i>Capsicum</i> seeds. Data were extracted for 2018-2020 (Table 1 of Annex 5) and show trade of vegetable seeds from countries with confirmed records* of the virus to EU countries. In 2020:</p> <table border="1" data-bbox="293 938 983 1219"> <thead> <tr> <th>Country of dispatch</th> <th>Tonnes (rounded figures)</th> </tr> </thead> <tbody> <tr> <td>USA</td> <td>3200</td> </tr> <tr> <td>China</td> <td>2000</td> </tr> <tr> <td>Czech Rep.</td> <td>350</td> </tr> <tr> <td>India</td> <td>160</td> </tr> <tr> <td>Brazil</td> <td>40</td> </tr> <tr> <td>Mexico</td> <td>7</td> </tr> <tr> <td>Iran</td> <td>1</td> </tr> </tbody> </table> <p>* Note that the virus may have a wider distribution than currently known (see section 6).</p>	Country of dispatch	Tonnes (rounded figures)	USA	3200	China	2000	Czech Rep.	350	India	160	Brazil	40	Mexico	7	Iran	1
Country of dispatch	Tonnes (rounded figures)																
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¹² Tomato hybrids are reported to have better vigour, uniformity, disease resistance and stress tolerance, and to have desirable horticultural traits including early fruiting, longer shelf life and consistent yield.

¹³ Tomato hybrids are reported to have better vigour, uniformity, disease resistance and stress tolerance, and to have desirable horticultural traits including early fruiting, longer shelf life and consistent yield.

Pathway	Seeds of tomato and <i>Capsicum</i>									
Transfer to a host	<p>Transmission from seed to seedlings has not been studied. Some information is available for tobacco (Li <i>et al.</i>, 2020) but the EWG noted that these results need validation (see section 2.2).</p> <p>In general, low transmission rates to seedlings occur when tobamoviruses are present on the seeds. However, even a very low percentage of disease transmission can result in a small infection focus and this infection can then be spread very rapidly by mechanical contacts within the growing crop (Dombrovsky & Smith, 2017).</p> <p>As the viral load on seed coat (especially untreated seeds) can be high, handling of seeds and further manipulations of plants in a nursery by a worker could potentially lead to infections.</p> <p>Transfer is less likely if the cultivar is resistant. However, a small portion of the plants may still become infected (Betti <i>et al.</i>, 1997, as well as some examples and possible reasons developed in section 2.7).</p> <p>Tomato cultivars carrying the <i>Tm-2²</i> gene are generally resistant to ToMMV (see section 2.7) and <i>Capsicum</i> cultivars carrying <i>L1</i> or <i>L3</i> genes/alleles also appear to be resistant (no data for <i>L4</i>, but it is highly likely that <i>L4</i> also confers resistance to ToMMV) (information from Plantum, the Netherlands, January 2022).</p>									
Likelihood of entry and uncertainty	<p>The EWG rated separately tomato and <i>Capsicum</i> seed.</p> <table border="1" data-bbox="293 767 2175 1050"> <thead> <tr> <th data-bbox="293 767 1234 802">Seeds of tomato</th> <th data-bbox="1234 767 2175 802">Seeds of <i>Capsicum</i></th> </tr> </thead> <tbody> <tr> <td data-bbox="293 802 1234 1050"> <p>high likelihood</p> <ul style="list-style-type: none"> ToMMV has been found associated with seeds, and other tobamoviruses are known to be to be seed-borne part of the imported seeds is not expected to carry resistance to ToMMV (e.g. seeds of determinate tomato cultivars (intended to produce fruit for processing)) the rate of seed to seedling transmission is expected to be very low but high numbers of seeds are being used every season. </td> <td data-bbox="1234 802 2175 1050"> <p>moderate uncertainty</p> <ul style="list-style-type: none"> uncertainty of the distribution (under-reported) likelihood of association whether entry with seeds has already been successful in some countries (but not recorded to date) the volume of seeds traded of tomato varieties not carrying the <i>Tm-2²</i> gene lack of knowledge on seed to seedling transmission </td> </tr> </tbody> </table> <p>The EWG discussed whether the likelihood should be lower for ToMMV than for ToBRFV (which was rated as high with low uncertainty), because part of the seeds traded would carry the <i>Tm-2²</i> resistance gene, and therefore would be largely resistant to ToMMV, i.e. limiting entry, and because there have not been entry reports to the same extent as for ToBRFV. However, the EWG concluded that the confirmed association with tomato seeds in trade, and other factors expressed above justify a high rating (although the likelihood of entry was assessed to be lower for ToMMV than for ToBRFV, but not as low as to become moderate). The uncertainty is higher than for ToBRFV.</p> <table border="1" data-bbox="293 1326 2175 1385"> <thead> <tr> <th data-bbox="293 1326 1234 1361">Seeds of <i>Capsicum</i></th> <th data-bbox="1234 1326 2175 1361">Seeds of <i>Capsicum</i></th> </tr> </thead> <tbody> <tr> <td data-bbox="293 1361 1234 1385">moderate likelihood</td> <td data-bbox="1234 1361 2175 1385">high uncertainty Same factors as for tomato, with even less data available for <i>Capsicum</i></td> </tr> </tbody> </table>		Seeds of tomato	Seeds of <i>Capsicum</i>	<p>high likelihood</p> <ul style="list-style-type: none"> ToMMV has been found associated with seeds, and other tobamoviruses are known to be to be seed-borne part of the imported seeds is not expected to carry resistance to ToMMV (e.g. seeds of determinate tomato cultivars (intended to produce fruit for processing)) the rate of seed to seedling transmission is expected to be very low but high numbers of seeds are being used every season. 	<p>moderate uncertainty</p> <ul style="list-style-type: none"> uncertainty of the distribution (under-reported) likelihood of association whether entry with seeds has already been successful in some countries (but not recorded to date) the volume of seeds traded of tomato varieties not carrying the <i>Tm-2²</i> gene lack of knowledge on seed to seedling transmission 	Seeds of <i>Capsicum</i>	Seeds of <i>Capsicum</i>	moderate likelihood	high uncertainty Same factors as for tomato, with even less data available for <i>Capsicum</i>
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Seeds of <i>Capsicum</i>	Seeds of <i>Capsicum</i>									
moderate likelihood	high uncertainty Same factors as for tomato, with even less data available for <i>Capsicum</i>									

Pathway	Seeds of tomato and <i>Capsicum</i>	
	Same factors as for tomato, but the likelihood of association is assessed to be lower (fewer outbreaks reported on <i>Capsicum</i> , only infected crops reported from China, and fewer interceptions than on tomato seeds)	
	The EWG notes that the PRA on ToBRFV rated only <i>Capsicum</i> with <i>L</i> resistance gene/allele, while all <i>Capsicum</i> seeds are rated here, and the ratings cannot be compared.	

8.1.2 Plants for planting (except seeds and pollen) of tomato and *Capsicum*

Table 4. Plants for planting (except seeds and pollen) of tomato and *Capsicum*

Pathway	Plants for planting (except seeds and pollen) of tomato and <i>Capsicum</i>
Coverage	<ul style="list-style-type: none"> Plants for planting of tomato and <i>Capsicum</i>, grafted or not. This pathway does not include seeds (see section 8.1.1) and pollen (see section 8.2), but includes seedlings, rootstocks and scions.
Plants concerned	<i>S. lycopersicum</i> , <i>Capsicum</i> spp.
Pathway prohibited in the PRA area?	<p>Partly.</p> <p>In the EU, import of plants of Solanaceae intended for planting, other than seeds, is prohibited from third countries, other than European and Mediterranean countries¹⁴, according to Annex VI point 18 of Regulation (EU) 2019/2072 (EU, 2019). The prohibition therefore cover all countries where ToMMV has been found to date (2021-10) except EU countries. The same regulations apply for the UK.</p> <p>The EWG had no information on the requirements of other non-EU EPPO countries.</p>
Pathway subject to a plant health inspection at import?	<p>Yes, at least in some EPPO countries.</p> <p>For example, in the EU and the UK, plants for planting other than seeds should be accompanied with a phytosanitary certificate and should be inspected at import. However, all non-EU countries where ToMMV is present are covered by the prohibition above.</p>
Pest already intercepted?	No interceptions reported for the EU or the UK on plants for planting other than seeds.
Most likely stages that may be associated	The virus may be present in the plant, as well as in the growing media associated with the plants.
Important factors for association with the pathway	<p>Tomato cultivars carrying <i>Tm-2²</i> are generally resistant and plants are unlikely to become infected under practical conditions. Infection of some <i>Tm-2²</i> plants has been observed under experimental conditions especially at elevated temperatures, and in one country in the field. <i>Capsicum</i> cultivars carrying <i>L1</i>, <i>L3</i> and/or <i>L4</i> are likely resistant (see section 2.7).</p> <p>Transplants are produced from seeds. The use of high-quality seeds certified as disease free is essential to reduce the risk of association with the pathway.</p>

¹⁴ Albania, Algeria, Andorra, Armenia, Azerbaijan, Belarus, Bosnia and Herzegovina, Canary Islands, Egypt, Faeroe Islands, Georgia, Iceland, Israel, Jordan, Lebanon, Libya, Liechtenstein, Moldova, Monaco, Montenegro, Morocco, North Macedonia, Norway, Russia (only the following parts: Central Federal District (Tsentralny federalny okrug), Northwestern Federal District (Severo-Zapadny federalny okrug), Southern Federal District (Yuzhny federalny okrug), North Caucasian Federal District (Severo-Kavkazsky federalny okrug) and Volga Federal District (Privolzhsky federalny okrug)), San Marino, Serbia, Switzerland, Syria, Tunisia, Turkey and Ukraine

Pathway	Plants for planting (except seeds and pollen) of tomato and <i>Capsicum</i>
	<p>Tomato transplants are usually produced in trays. This is probably similar for pepper transplants. Production techniques and practices of transplants may influence the risk of association with the pathway:</p> <p>1 - Grafting increases the probability of association with the plants for planting</p> <p>Grafting of tomato transplants (Figure 4) has become an important cultivation practice for greenhouse tomato fruit production systems (Singh <i>et al.</i>, 2017). Limited information on compatibility with open-field tomato cultivars is available but vegetable grafting is also gaining interest in open-field and high tunnel tomato production (OECD, 2017).</p> <p>The grafting technique is widely used on a commercial scale (e.g. in Spain, nearly 90% of tomato plants are grafted (Penella <i>et al.</i>, 2017); in Italy, 40% of the plants used for the commercial tomato production are grafted (Tomassoli, pers. comm., 2019 cited in EPPO, 2020b), whereas only 12% are grafted in Turkey (Yetişir, 2017)). Grafting is mainly performed to improve the vigour of the plant. There are a variety of grafting techniques, but the most widely adopted method worldwide for grafted tomato production is tube grafting (OECD, 2017). Tomato is grafted on different species (e.g. <i>S. lycopersicum</i>, <i>S. melongena</i>, <i>S. lycopersicum</i> × <i>S. habrochaites</i>) (GEVES, 2019; Yetişir, 2017). Pepper is less commonly grafted (e.g. In Italy, only 10% of the pepper plants are grafted (Tomassoli, pers. comm., 2019 cited in EPPO, 2020b)) because of the lack of interesting rootstocks. Grafting of pepper is mainly performed on rootstocks of the same species, <i>C. annuum</i>.</p> <p>Transmission of diseases in the production process of grafted seedlings occurs more easily than in regular seedling production. This is because some diseases can be transmitted from seedling to seedling by cutting tools. For CGMMV, the use of grafting knives can infect the four following grafted plants (Reingold <i>et al.</i>, 2015). In addition, the cut surfaces of both the rootstock and the scion are entry points for pathogens, and high relative humidity and ambient temperature in the healing chambers promote the spread of diseases (Yetişir, 2017). Handling during the grafting process is also an important factor. Grafting is an additional possible source of infection by ToMMV.</p> <p>2 – Plantlets are likely to be symptomless</p> <p>Symptoms on non-grafted young plantlets would probably not be visible in the plant production nursery (section 2.5) because they have a short production period. Grafted plants have a longer production time (they are grown for one month longer according to EPPO, 2020b), but it is still uncertain whether ToMMV symptoms would be visible before they are traded.</p> <p>3 – Water supply increases the probability of association with the plants for planting.</p>

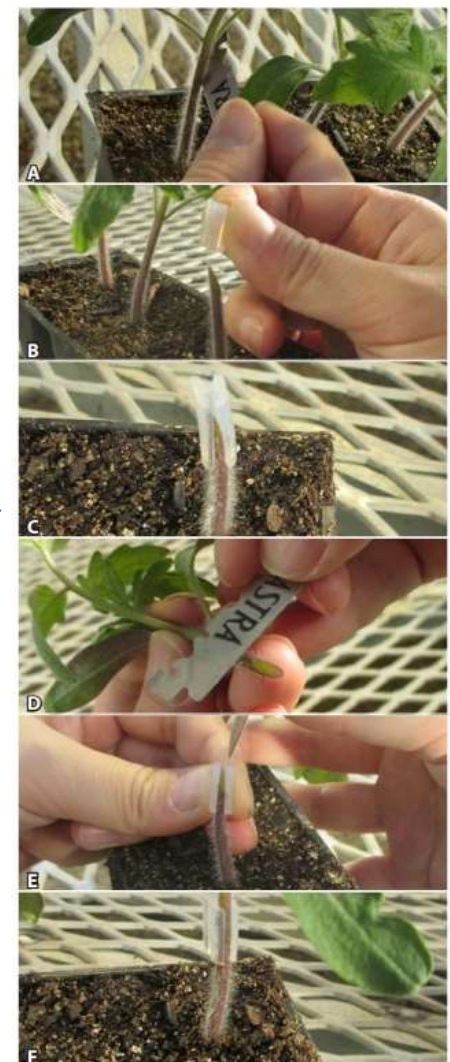


Figure 4. The procedure for splice grafting tomato plants (Guan & Hallett, 2016).

Pathway	Plants for planting (except seeds and pollen) of tomato and <i>Capsicum</i>			
	<p>Containerized production of tomato transplants is usually performed with abundant supply of quality water. Irrigation is performed with overhead (usually with an automated watering system) and ebb and flow (or ebb and flood). When producing transplants, water may be chlorinated (1 ppm chlorine) (APS, 2014) but this is probably not sufficient to inactivate tobamoviruses (Li <i>et al.</i>, 2015). It is assumed to be the same for pepper transplant production. Aerial irrigation can cause injury to plants that facilitate infections and circulating water can be a source of infection (Section 2.2).</p> <p>4 - The re-use of trays increases the probability of association with the plants for planting. Transplant trays may be cleaned and sterilized before reuse to avoid the build-up of soilborne pathogens. When not appropriately cleaned and sterilized, the re-use of transplant trays is a source of infection by contact, which increases the probability of association with the plants for planting.</p>			
Survival during transport and storage	Conditions that allow survival of the plants will also allow survival of ToMMV.			
Trade	<p>In the EU, transplants used to establish fruiting crops in a country are often grown in the same country. In some European countries however, plants are introduced from other EU countries, mainly originating from the Netherlands (Werkman & Sansford, 2010). The plants exported from the Netherlands would be (mostly) from resistant cultivars and intended for greenhouse production. For example, most of plants imported to the United Kingdom and Belgium are produced in the Netherlands. In the Rheinland (west part of Germany, most important area for German tomato production), most tomato and pepper plants for planting are imported from the Netherlands (except plants for organic production) (Scholz-Döbelin, pers. comm. cited in EPPO, 2020b). In Austria, commercial tomato growers buy in general young plantlets from abroad. Local production of plants (from seeds) is only for the hobby sector (Steffek, pers. comm., 2020 cited in EPPO, 2020b). One company in the Netherlands (Beekenkamp) produces annually over 600 million young plants of tomato, pepper and other vegetables in more than 30 hectares of greenhouses (https://www.beekenkamp.nl/plants/en/tomato-nursery/). Tens of thousands of tomato and pepper plants for planting from Spain are exported every year to France and Portugal (Guitian-Castrillon, pers. comm., 2019 cited in EPPO, 2020b), as well as a more limited number of tomato plants from the United Kingdom to France (Gentit, pers. comm., 2019 cited in EPPO, 2020b).</p> <p>For field crop for processing industry (i.e. susceptible cultivars), it is not known if there is a trade from countries where the virus occurs.</p>			
Transfer to a host	Host plants for planting are directly introduced at production sites.			
Likelihood of entry and uncertainty	<p>The EWG rated separately tomato and <i>Capsicum</i> plants for planting.</p> <table border="1"> <tr> <td> <p>Plants for planting of tomato (except seeds and pollen)</p> <p>moderate likelihood</p> <ul style="list-style-type: none"> • Young plants of tomato are imported in lower quantities than seeds. • A large part of imported plants, especially for the production of fresh fruit for consumption, would carry the <i>Tm-2²</i> resistant gene (plants for planting of susceptible cultivars are probably produced locally). • Easily mechanically transmitted which increases the likelihood that plants become infected if the virus is present in the area. </td> <td> <p>moderate uncertainty</p> <ul style="list-style-type: none"> • uncertainty about the worldwide distribution of the virus (probably under-reported) • limited trade data, especially on susceptible cultivars </td> </tr> </table>		<p>Plants for planting of tomato (except seeds and pollen)</p> <p>moderate likelihood</p> <ul style="list-style-type: none"> • Young plants of tomato are imported in lower quantities than seeds. • A large part of imported plants, especially for the production of fresh fruit for consumption, would carry the <i>Tm-2²</i> resistant gene (plants for planting of susceptible cultivars are probably produced locally). • Easily mechanically transmitted which increases the likelihood that plants become infected if the virus is present in the area. 	<p>moderate uncertainty</p> <ul style="list-style-type: none"> • uncertainty about the worldwide distribution of the virus (probably under-reported) • limited trade data, especially on susceptible cultivars
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Pathway	Plants for planting (except seeds and pollen) of tomato and <i>Capsicum</i>	
	<ul style="list-style-type: none"> • Grafted plants are widely used and grafting increases the likelihood of spread at young plant nurseries. • Import of plants for planting of tomato other than seeds is prohibited in many EPPO-countries but the virus is already present in the EPPO-region. • No records found of ToMMV on young plants. The virus may occur in young plants in China (this is not specified in the literature available), but young plants are probably not imported from China and would be prohibited in many EPPO-countries. 	
<p>Unlike for ToBRFV, there are no reported outbreaks linked to plants for planting, which lowered the likelihood compared to ToBRFV.</p>		
	<p>Plants for planting of <i>Capsicum</i> (except seeds and pollen)</p> <p>moderate likelihood</p> <ul style="list-style-type: none"> • Young plants of <i>Capsicum</i> are imported in lower quantities than seeds • A large part of imported plants would carry <i>L</i> resistance genes (plants for planting of susceptible cultivars probably produced locally) • Easily mechanically transmitted which increases the likelihood that plants become infected if the virus is present in the area. • Grafted plants are not used (see tomato) • Import of plants for planting of <i>Capsicum</i> and other Solanaceae other than seeds is prohibited in many EPPO-countries but the virus is already present in the EPPO-region. • No records found of ToMMV on young plants. There may be findings in young plants in China but young plants are probably not imported from China, and would be prohibited in some EPPO-countries. 	<p>High uncertainty</p> <ul style="list-style-type: none"> • Worldwide distribution of the virus (probably under-reported) • Except from China, very few findings of the virus have been reported in <i>Capsicum</i> crops. • Limited trade data.

8.1.3 Fruit of tomato and *Capsicum*

Table 5. Fruits of tomato and *Capsicum*

Pathway	Fruit of tomato and <i>Capsicum</i>
Coverage	<p>Fruits of Solanaceae hosts are sold for the fresh market or processed into many different products (puree, paste, peeled, canned, chutney, ketchup, juice, dried etc.). This pathway focuses on host fruits for the fresh market and includes containers (e.g. trays, boxes and packaging) when associated with fruits. The likelihood of entry through processed and dried host fruit is assessed to be very low (see section 8.2).</p> <p>Tomatoes intended for processing are not likely to be traded internationally in a fresh state to be processed in another country. Such tomatoes are a low value product. Once harvested at the right ripeness, processing tomatoes need to be processed rapidly (6 hours; Petti et al., 2022; Boriss & Brunke, 2005). Therefore, it would not be economical nor feasible to import such tomatoes, except at short distance (e.g. production and processing in adjacent areas of two countries). No information was sought for <i>Capsicum</i>; <i>Capsicum</i> for industrial purposes are covered by separate commodity codes (see Trade below). Processing tomatoes and <i>Capsicum</i> were not considered when studying this pathway.</p>
Plants concerned	<i>S. lycopersicum</i> , <i>Capsicum</i> spp.
Pathway prohibited in the PRA area?	No
Pathway subject to a plant health inspection at import?	<p>Yes, at least in some EPPO countries.</p> <p>For example, in the EU and the UK, a phytosanitary certificate is required to import fruits of Solanaceae from third countries. However depending on the origin, a reduced frequency check may be applied (https://ec.europa.eu/food/sites/food/files/plant/docs/ph_biosec_trade-non-eu_prods-recom-reduced-ph-checks_2019.pdf; https://planthealthportal.defra.gov.uk/eu-exit-guidance/imports/reduced-physical-checks-regime/).</p>
Pest already intercepted?	No. No known interception.
Most likely stages that may be associated	ToMMV may be found in all plant parts, including fruit (and pods). It may also remain infectious on packaging material that comes in contact with infected plants or fruits.
Important factors for association with the pathway	<p>Symptoms have been recorded on tomato fruit (section 2.6). The presence of ToMMV in fruit is expected at least for susceptible tomato and <i>Capsicum</i> genotypes.</p> <p>For the related ToBRFV and other tobamoviruses, high virus concentrations have been reported in fruit (Dombrovsky <i>et al.</i>, 2017; Klap <i>et al.</i>, 2020; Salem <i>et al.</i>, 2020) (cited in Dutch NPPO, 2020).</p> <p>The cultivars used for fresh fruit production indoors are most likely to carry the <i>Tm-2²</i> gene (tomato) or <i>L1</i>, <i>L3</i> and <i>L4</i> (<i>Capsicum</i>).</p> <p>Generally, there are very few reports on outbreaks of ToMMV in fruit crops worldwide. Such outbreaks are known from China, Brazil and Mauritius. However, the type of crop found infected in the USA, Iran, India and Mexico is not detailed in the literature. In the Czech Republic and Israel, ToMMV was associated only with seed crops and in Spain with research material.</p>

Pathway	Fruit of tomato and <i>Capsicum</i>										
	<p>Fruits from infected crops are likely to be infected:</p> <p>1 - Manipulations increase the risk of association with fruits. Association with fruits is likely to be higher for indoor production compared to outdoor production. All manipulations during fruit production (plant tying, pruning, removing of plant suckers), harvesting (e.g. the use of manual or mechanical harvesting), will increase the likelihood of association with the pathway, because of the likelihood of mechanical transmission from plant to plant. As more manipulations are expected for intensive indoor production rather than for outdoor production, the likelihood of association with fruit is considered higher for indoor production.</p> <p>2 – Sorting out process When symptoms are visible, fruits will be downgraded and may not be traded anymore for fresh consumption (i.e. rather processed for use as tomato sauce, paste etc.). However, asymptomatic fruits are still likely to be present in the consignment and infection will not be evident at inspection.</p> <p>3 – Cultivation of seed trial lines close to fruit production. Trial lines may be subject to more limited seed testing than commercial varieties (Dombrovsky, pers. comm., 2019 cited in EPPO, 2020b). Transfer could occur because of the cultivation of trial lines or trial lots of tomato seed close to fruit production. Fruit production businesses and seed businesses collaborate to grow trial lines to determine their suitability. Tomato trial lines are usually grown at the same time and in the same place as larger fruit production crops. Van Brunschot <i>et al.</i> (2014) have reported trial lines infected with tospoviruses. Therefore, trial lines can be a source of infection for tomato crops (AGDAWR, 2018). In such case, infection of fruits would require a combination of different pathways (e.g. seed infection of the trial seeds and mechanical transmission).</p>										
Survival during transport and storage	<p>As the pathogen is being transported in living plant material, there is a high probability that the virus will survive transport and storage. The virus may also survive during transport and storage on packaging material.</p>										
Trade	<p>- Tomatoes. Eurostat data (i.e. imports into EU countries) for 2018-2020 show little import of tomatoes (07020000 Tomatoes, fresh or chilled; Table 2, Annex 5) from countries with confirmed records* of the virus to EU countries. It is noted that during the period 2001-2010, Mexico exported a maximum of 2785 tonnes/year to EPPO countries (data from FAOSTAT analysed by EPPO, 2015), and that there appears to have been a significant decrease of imports to the EU.</p> <table border="1" data-bbox="327 1106 945 1284"> <thead> <tr> <th>country</th> <th>Tonnes (rounded figure)</th> </tr> </thead> <tbody> <tr> <td>Czech Rep</td> <td>4500</td> </tr> <tr> <td>Iran</td> <td>1</td> </tr> <tr> <td>Mexico</td> <td><1</td> </tr> <tr> <td>USA</td> <td><1</td> </tr> </tbody> </table> <p>i.e. no trade reported from China, Brazil and Mauritius, where the only records in production are. * Note that the virus may have a wider distribution than currently known (see section 6).</p> <p>The highest trade volume is from Czech Republic but in this country the virus has only been reported in seed crops and not in fruit crops.</p>	country	Tonnes (rounded figure)	Czech Rep	4500	Iran	1	Mexico	<1	USA	<1
country	Tonnes (rounded figure)										
Czech Rep	4500										
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USA	<1										

Pathway	Fruit of tomato and <i>Capsicum</i>																																				
	<p>- <i>Capsicum</i>. Eurostat data (i.e. imports into EU countries) for 2018-2020 were extracted for fruits of: sweet peppers (codes 07096010 Fresh or chilled sweet peppers, Table 3, Annex 5) and a category that covers other <i>Capsicum</i> (07096099 Fresh or chilled fruits of genus <i>Capsicum</i> or <i>Pimenta</i> (excl. for industrial manufacture of capsaicin or capsicum oleoresin dyes, for industrial manufacture of essential oils or resinoids, and sweet peppers); Table 4, Annex 5), but this category covers not only other <i>Capsicum</i>, but also <i>Pimenta</i>. The data show some imports from countries with confirmed records* to the EU. For 2020:</p> <table border="1" data-bbox="331 359 1832 710"> <thead> <tr> <th colspan="2">Fresh or chilled sweet peppers* Tonnes (rounded figures)</th> <th colspan="2">Fresh or chilled fruits of genus <i>Capsicum</i> or <i>Pimenta</i> (see above for excluded commodities). Tonnes (rounded figures)</th> </tr> </thead> <tbody> <tr> <td>Czech Rep.</td> <td>6400</td> <td>India</td> <td>650</td> </tr> <tr> <td>India</td> <td>170</td> <td>Czech Rep.</td> <td>260</td> </tr> <tr> <td>Mexico</td> <td>7.5</td> <td>Mexico</td> <td>70</td> </tr> <tr> <td>USA</td> <td><1</td> <td>China[^]</td> <td>16</td> </tr> <tr> <td>Brazil[#]</td> <td><1</td> <td>USA</td> <td>1</td> </tr> <tr> <td>China[^]</td> <td>10</td> <td>Iran</td> <td><1</td> </tr> <tr> <td>Iran[^]</td> <td>13</td> <td>Brazil[^]</td> <td><0.1</td> </tr> <tr> <td>Mauritius[^]</td> <td><1</td> <td></td> <td></td> </tr> </tbody> </table> <p>[^] Data from 2019, no data from 2020. [#] data from 2018, no data from 2019 and 2020 * Note that the virus may have a wider distribution than currently known (see section 6).</p>	Fresh or chilled sweet peppers* Tonnes (rounded figures)		Fresh or chilled fruits of genus <i>Capsicum</i> or <i>Pimenta</i> (see above for excluded commodities). Tonnes (rounded figures)		Czech Rep.	6400	India	650	India	170	Czech Rep.	260	Mexico	7.5	Mexico	70	USA	<1	China [^]	16	Brazil [#]	<1	USA	1	China [^]	10	Iran	<1	Iran [^]	13	Brazil [^]	<0.1	Mauritius [^]	<1		
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Mauritius [^]	<1																																				
Transfer to a host	<p>Release of sap from vine tomatoes during handling is likely to occur and therefore contaminate containers and equipment (Dombrovsky, pers. comm., 2019 cited in EPPO, 2020b). Contamination of containers or other fruits by the fruit themselves will only occur via damaged fruits where sap is released. The presence of the tomato peduncle may also cause damage to other fruits during grading, which may release sap. Peppers are always sold with a peduncle. Pepper fruit are less susceptible to physical damage and consequential sap release than ripe tomato fruit.</p> <p>When imported fruit is stored or repacked at destination in facilities that also grow tomatoes, consignments can be present for several days at packing stations. In that specific case, ToMMV may be mechanically transmitted from imported infected fruits to a suitable host plant if workers and machinery are shared between the packing area and the production site. The practice of using the same facilities for imported fruit and for production was shown as a factor facilitating transfer of pests in the past in tomato and pepper supply chains (e.g. PepMV, <i>Tuta absoluta</i>, <i>Thaumatotibia leucotreta</i>) (EPPO RS, 2010). This may be changing as a result of these recent pest introductions but there is no evidence of this for the whole EPPO region (EPPO, 2015). Because of ToBRFV, packing and sorting stations, at least in some EPPO countries (e.g. EU, UK), take strict hygiene measures to prevent the spread of ToBRFV through packing stations (e.g. cleaning and disinfection of crates before they are returned to tomato producers) (https://ec.europa.eu/food/audits-analysis/country_profiles/details.cfm?co_id=NL; https://planthealthportal.defra.gov.uk/assets/uploads/Tomato-brown-rugose-fruit-virus-contingency-plan-v7.pdf). These measures will also be effective against other tobamoviruses, and transfer would therefore be unlikely. However, it is not known how strictly these measures are applied, and whether all EPPO countries apply such measures. Finally, if the regulatory status of ToBRFV changes, it is uncertain if such practices will be maintained by all countries/growers.</p> <p>Another possibility allowing transfer, is ToMMV contamination of workers hands with infested fruits (bought e.g. in a supermarket), when workers are involved in a place of production of host plants for planting or fruit. This can only be avoided if strict hygiene measures are applied on site.</p>																																				

Pathway	Fruit of tomato and <i>Capsicum</i>
	<p>Finally, fruits imported for consumption may be inappropriately used for propagation by amateur gardeners or small professional farmers. Fruits could also be discarded or partially composted, and the fruits with seeds can germinate (volunteers). In each case the resulting plants may be infected. However, this is more likely to occur in domestic situations and not in major crop production sites.</p> <p>Imported tomato fruits can also be repacked at destination in facilities that also pack local fruits, and transfer may occur via contaminated material (e.g. containers of imported tomato fruits reused locally in tomato production sites to harvest local tomatoes).</p>
Likelihood of entry and uncertainty	<p>Fruits of tomato and <i>Capsicum</i>: low likelihood with moderate uncertainty</p> <p>Low likelihood because of the low likelihood of association with imported fruit:</p> <ul style="list-style-type: none"> - Except from China, few outbreaks have been reported in tomato and <i>Capsicum</i> fruit crops - Low import volume from countries where the virus has been reported in fruit crops - Many cultivars used for fresh fruit production carry resistance - Hygiene measures at packing stations used because of ToBRFV will also be effective against ToMMV <p>Uncertainty</p> <ul style="list-style-type: none"> - Distribution of ToMMV worldwide - Whether resistant cultivars are used in the countries from which fruit is imported - How strictly hygiene measures are applied at packing stations and whether these measures will be continued if ToBRFV would be deregulated <p>The EWG did not rate different fruit pathways. A distinction can be made between ‘fruit stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local fruits’ and ‘fruits already packed for final consumer before export’. As for ToBRFV, the second pathway (fruits already packed for final consumer before export) is assessed to be an unlikely pathway of entry (very low likelihood with low uncertainty).</p>

8.1.4 Used containers, tools, equipment and conveyance vehicles associated with the hosts production and supply chain

Note: Containers used to transport fruits and trays used to transport plants for planting are considered together with the fruit and plants for planting pathways.

Tobamovirus may remain viable for a long time on surfaces. International movement of the virus and transfer to host plants is likely to happen only if the containers, tools and equipment have previously been associated with the production and supply chain for hosts.

Used containers could be moved without fruits between countries, e.g. Europool System is moving more than one billion trays a year which are not owned by users but rented¹⁵ (Europool System, 2019). Containers provided by Europool System are cleaned, disinfected and tested using a validated protocol for ToBRFV, which would presumably also be effective against ToMMV. However, such protocols are probably not applied for all used containers, tools, equipment and conveyance vehicles associated with the hosts' production and supply chain.

Because of ToBRFV, at least in some EPPO countries (e.g. EU, UK), strict hygiene measures have been implemented to prevent the spread of ToBRFV with containers, tools or equipment (https://ec.europa.eu/food/audits-analysis/country_profiles/details.cfm?co_id=NL; https://ec.europa.eu/food/audits-analysis/country_profiles/details.cfm?co_id=NL; <https://planthealthportal.defra.gov.uk/assets/uploads/Tomato-brown-rugose-fruit-virus-contingency-plan-v7.pdf>; <https://planthealthportal.defra.gov.uk/assets/uploads/Tomato-brown-rugose-fruit-virus-contingency-plan-v7.pdf>). These measures will also be effective against other tobamoviruses. The likelihood of transfer would be lower than for ToBRFV because there are fewer susceptible cultivars. However, there is a high uncertainty: it is not known how strictly these measures are applied, and whether all EPPO countries apply such measures; the volume of movement on this pathway is not known.

Likelihood of entry: low; *Uncertainty:* high

¹⁵Videos explaining how recycled containers are provided by Europool System are available at <https://youtu.be/JFxY5mOqKHc> and at https://www.youtube.com/watch?v=L__BBxSoEzk.

8.2 Unlikely pathways: very low likelihood of entry

- **Travellers (persons working in place of production of host plants)**

Persons working in a place of production may have been in contact with infected or contaminated material (e.g. seasonal workers). Persons installing and fixing greenhouses as well as trellising workers may travel internationally and were suspected to be a source of contamination for PepMV in the EU. Other people, such as technical teams, experts and crop advisors traveling around and visiting different places of production can introduce ToMMV. It is thought that one of the main reasons for the rapid dissemination of ToBRFV in Israel was the very large number of people who visited the first outbreak (Dombrovsky, pers. comm., 2019 cited in EPPO, 2020b). Such persons may contribute to the entry of the virus if they travel to another country, but may contribute to its spread within and between production sites within the same area (locally or regionally) (see section 11). Since the emergence of ToBRFV, workers moving between places of production are taking more precautions, and this pathway is considered as less likely for ToMMV. However, it is not known how strictly such precautions are applied, and whether it is common practice in all EPPO countries. In addition, few outbreaks of ToMMV have been reported in production to date.

Uncertainty: moderate

- **Natural spread, from countries where ToMMV occurs to EPPO countries where it does not occur (over the next 10 years in absence of eradication/control measures).**

Even if insects present in an infected production site may have the capacity to fly long distances (e.g. more than 10 km in search of desirable floral rewards for *Apis mellifera*), it is assumed that only a small number of these insects (e.g. ‘scout’ bees) fly longer distances, and it is well known that bees tend to forage within 2.0 km of their hive if there are attractive floral resources in the vicinity (Hagler *et al.*, 2011). Other tobamoviruses are known to be transmitted by other animals such as birds (Broadbent, 1976; Peters *et al.*, 2012). Like for pollinating insects, it is assumed that their role will be limited compared to human-assisted pathways between different production areas. Natural spread from these countries to the rest of the EPPO region via pollinating insects and other animals is therefore considered to be an unlikely pathway.

Uncertainty: low

- **Pollinating insects (from third countries) used in host fruit production**

No specific information was found for ToMMV, but Australian Government (2019) mention bees as a possible mode of transmission. Transmission of ToMMV by pollinating insects would be unlikely for tomato cultivars carrying the *Tm-2²* resistance gene. *Capsicum* are self-pollinating, and pollinating insects are not a pathway.

Tomato flowers are typically pollinated by wind and/or bees in open fields and bumblebees in greenhouses or screenhouses. Before the 1990s, pollination was performed in tomato greenhouse conditions mechanically by vibrating the plants or with hormones. Nowadays, pollination in greenhouse tomato crops is commonly performed with bumblebees (Velthuis & van Doorn, 2006).

Tomato crops under greenhouse is the main agricultural crop that bumblebees pollinate. Worldwide, this involves about 95% of all bumblebee sales. Up to 50 bumblebee colonies are used per hectare of fruit production during the growing season (Velthuis & van Doorn, 2006). Import of pollinating insects (including bumblebees) is often restricted in the EPPO region. E.g. in the European Union, imports of bees are restricted in order to prevent the introduction of two exotic pests, which are absent from the EU, the small hive beetle (*Aethina tumida*) and the Tropilaelaps mite (*Tropilaelaps* spp.). Only queen honeybees (*Apis mellifera*) and bumblebees (*Bombus* spp.) can be imported from a list of third countries which includes Israel, Mexico and the USA (*Bombus* only) (EU, 2020, 2021). Most of the market share is covered by three companies (Biobest (BE), Koppert Biological Systems (NL) and Bunting Brinkman Bees (NL)), having rearing facilities not only in their homeland, but also in other countries and on other continents, usually under their own name (Velthuis & van Doorn, 2006).

Movement within the EU is possible with a health certificate. Although a large quantity of commercially produced bumblebees is moved within the PRA area, the likelihood of movement of ToMMV with traded bumblebees is estimated as very low because the probability of association is very unlikely. This is because bumblebees are produced on a diet of bee-collected pollen and sugar water (Velthuis & van Doorn, 2006;

Werkman & Sansford, 2010). Therefore, the movement of pollinating insects are not considered to pose a risk of entry into the PRA area from distant third countries. The main risk from bumblebees is associated with spread within an infected area.

Uncertainty: low

- **Soil and growing media on its own**

This pathway covers growing media with or without a component of organic material.

The virus may be present in soil and growing media if infected plants have been in contact with them. Transfer to the roots of a host plant is possible through soil and plant debris: the infectivity of tobamoviruses is preserved in plant debris and in contaminated soil for months to years (Dombrovsky & Smith, 2017; Smith & Dombrovsky, 2019).

The import of soil and growing media is regulated in many EPPO countries (e.g. soil and growing media as such from all third countries other than Switzerland, cannot be imported in the EU according to Annex VI point 19 and 20 of Commission Implementing Regulation (EU) 2019/2072 (EU, 2019)). If soil or growing media in which infected plants were previously grown is imported, it may be a pathway for entry of the pest. However, this is considered very unlikely for growing media for professional use. When re-using growing media for tomato production, this would be done within a production site or locally. It is very unlikely that tomato and *Capsicum* growers will reuse growing media that has already been used in another country or by another producer.

Uncertainty: low

- **Pollen of host plants**

There is no evidence that pollen can be a pathway for ToMMV. For tobamoviruses, there is one experimental report of pollen transmission for CGMMV (see section 2.2). There are no known studies on transmission of ToMMV through pollen.

Uncertainty: moderate

- **Processed and dried fruits of hosts**

The virus is expected to survive in dried fruits or products made from tomato and *Capsicum* for several months. However, it is very unlikely that ToMMV will transfer from processed and dried fruit to commercial production of host crops. High temperatures in some processes may also denature the virus (e.g. canning). The same apply to processed and dried peas and common beans.

Uncertainty: low

- **Soil or growing media attached to non-host plants**

It is very unlikely that non-host plants for planting will be grown in artificial growing media previously used to grow hosts (i.e. ToMMV is very unlikely to become associated with such plants). It may be more likely that non-host plants are grown in a field on which a ToMMV-infected crop had previously been grown. However, the EWG considered the likelihood of transfer of ToMMV from soil attached to non-hosts to a ToMMV host plant to be very low. It is unlikely that such plants will be replanted in a field where host plants are grown (i.e. making transfer unlikely).

Uncertainty: low

The following pathways from the EPPO Secretariat’s tree of pathways have no relevance for ToMMV and its hosts: wood, bark and related commodities; underground plant parts, hitchhiking.

8.3 Overall rating of the likelihood of entry

<i>Rating of the overall likelihood of entry</i>	<i>Very low</i> <input type="checkbox"/>	<i>Low</i> <input type="checkbox"/>	<i>Moderate</i> <input type="checkbox"/>	<i>High</i> X	<i>Very high</i> <input type="checkbox"/>
<i>Rating of uncertainty</i>			<i>Low</i> <input type="checkbox"/>	<i>Moderate</i> X	<i>High</i> <input type="checkbox"/>

9. Likelihood of establishment outdoors in the PRA area

In the PRA area, ToMMV has only been detected outdoors in the Czech Republic, in three field seed crops. ToMMV has also been found outdoors in other countries, at least in China, Iran, and USA (Florida) (e.g. Li *et al.*, 2014, 2017; Alavi & Massumi, 2014; Webster *et al.*, 2014; Fillmer *et al.*, 2015). In Brazil and India, the reports do not specify if ToMMV was found indoors or outdoors.

9.1 Climatic suitability

The biological functions of viruses are strongly integrated with those of their host plants and there is no indication that their requirements in terms of environment are substantially different from those of their host plants.

Tomato, pepper, pea and common bean are grown in a wide range of climates throughout the PRA area. In the northern part of the PRA area, tomato and pepper are only grown commercially under active protected cultivation (with heating) (Section 10). However, they are also grown outdoors in domestic gardens in the summer. In warmer areas in the southern part of the PRA area, they may be commercially grown outdoors as well as under passive greenhouse (without heating). Pea and common bean are grown outdoors in most of the PRA area. Tobacco is grown in a limited part of the region, in Mediterranean to continental conditions with warm summers.

► Climatic conditions are not a limiting factor for the establishment of ToMMV in the PRA area and it is considered that ToMMV could establish wherever suitable hosts are grown.

9.2 Host plants in the EPPO region

Considering solanaceous hosts as well as pea and common bean, hosts of ToMMV are grown throughout the EPPO region, possibly except in northernmost areas.

The northern limit of host cultivation in the EPPO region is estimated to be ca. 63°N, based on information from Finland, the UK and Norway. In Finland, there are commercial crops of *Pi. sativum* up to about 63°N (LUKE, 2022). In the UK, pea crops are grown up to Dundee in Scotland (i.e. 56°N) (Duncan Allen, pers. comm., 2022-02). In Norway, bean crops are found in the south-eastern part of the country, South of Oslo (which is located at 60°N) and pea crops South of 59°19'N (Z. Hamborg, pers. comm., 2022-02).

Garden cultivation probably extends beyond the northern limits of commercial cultivation.

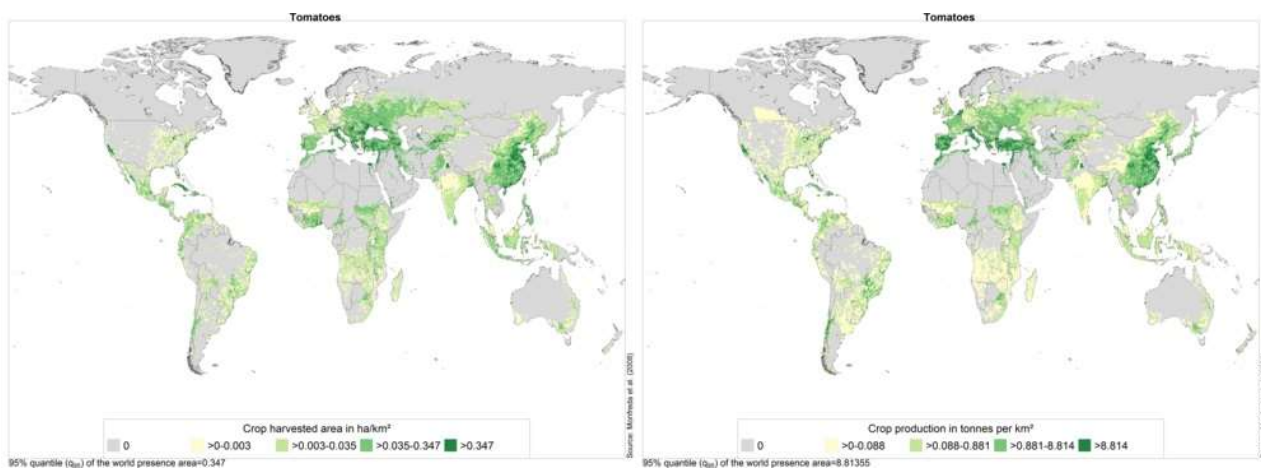
Tomato

Tomato (*S. lycopersicum*) is grown worldwide outdoors and under protected conditions (**Erreur ! Référence non valide pour un signet.**5), commercially and in gardens. Under cultivation, tomato is grown as an annual crop.

In countries in Northern and Central Europe, large quantities of tomato plants are cultivated, mostly in greenhouses for fruit production (Section 10). Furthermore, there are half-yearly outdoor cultivations in private gardens, on balconies or in private greenhouses (JKI, 2019). In southern Europe, fresh tomatoes can be produced in greenhouses or in open field (in Italy, approximately 10% of tomato is produced indoor, representing 7 229 hectares and 535 564 tonnes of fruit in 2018 (ISTAT database).

In part of the EPPO region, cultivars carrying the *Tm-2²* resistance gene are likely to be widely used in crops with a higher likelihood of tobamovirus transmission due to higher levels of hands-on management, i.e. especially for the production of fresh fruit for consumption in greenhouse or in the field, but not for the production of fruit for processing in the field (see section 2.7).

Figure 5. Tomato production in the world, both indoor and outdoor, in 2000 (left: harvested area in ha/km²; right: tonnes per km²) (Monfreda *et al.*, 2008)

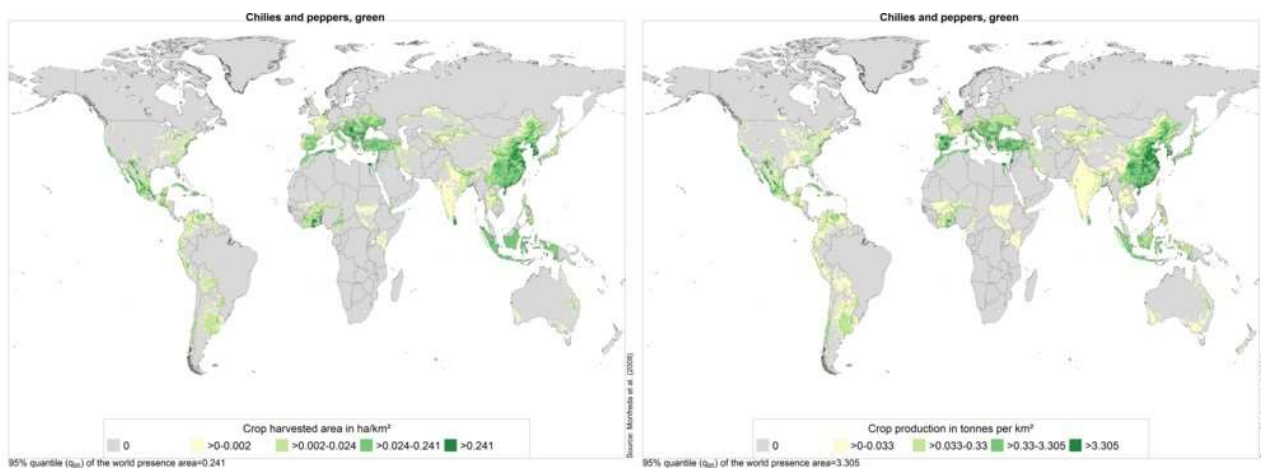


Pepper

Peppers (*Capsicum* spp.) are grown worldwide outdoors and under protected conditions (Figure 6), commercially and in gardens. In southern Europe, fresh peppers can be produced in greenhouses or in open field. In Italy for example, 30% of pepper is produced indoor, representing 1 976 hectares and 7 897 tonnes of fruit in 2018 (ISTAT database). In Spain, peppers are cultivated both in protected conditions (14 486 ha) and outdoors (6 743 ha). Mainly *L* resistant peppers are grown commercially in the EU and Israel. However, this may not be the case for varieties used by amateurs: when bought at garden centres/supermarkets for bioassays, pepper and hot pepper varieties used by amateurs were not found to harbour *L* resistance genes/alleles (Tomassoli, pers. comm., 2019 cited in EPPO, 2020b).

Peppers are grown for fresh fruit or for fruit for processing. For example, in 2019 Spain produced 1 399 192 tonnes of peppers, of which 153 054 tonnes were destined to processing (MAPA 2021).

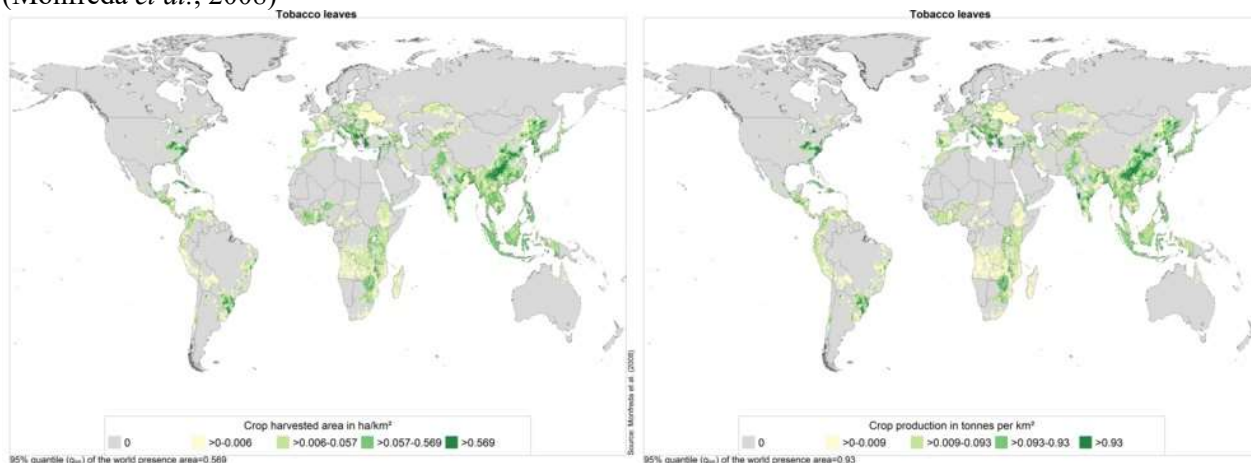
Figure 6. Chillie and pepper production in the world, both indoor and outdoor, in 2000 (left: harvested area in ha/km²; right: tonnes per km²) (Monfreda *et al.*, 2008)



Tobacco (*Nicotiana tabacum*)

In the EU in 2018, tobacco was grown on 66 000 ha (half as much as in 2001). In the EU, tobacco is grown in 12 countries, with the main producers being Italy, Spain, Poland, Greece, Croatia, France, Hungary and Bulgaria (together 99% of the EU tobacco production). (https://ec.europa.eu/info/food-farming-fisheries/plants-and-plant-products/plant-products/tobacco_en). Turkey, Uzbekistan, Azerbaidjan, Tunisia, Ukraine, Bosnia, Albania, Kazakhstan, Morocco, Kyrgystan, Moldova, Tadjikistan are also producers (<https://beef2live.com/story-ranking-countries-produce-tobacco-263-212893>).

Figure 8. Tobacco production in the world, in 2000 (left: harvested area in ha/km²; right: tonnes per km²) (Monfreda *et al.*, 2008)



Common bean (*Phaseolus vulgaris*) and pea (*Pisum sativum*)

Ph. vulgaris and *P. sativum* are cultivated commercially and grown in gardens throughout the EPPO region. Pea and common beans are probably mostly grown outdoors, although seed crops may be grown in protected conditions, and some fruit production may also be conducted in protected conditions. For example, in Spain, there is a limited area of green beans (1 525 ha) and peas (113 ha) grown in greenhouses (MAPA, 2021) to protect the crop from high temperatures and for getting early production.

Other plants

The known host weed *Chenopodium murale* is widespread in the EPPO region and could possibly serve as reservoir for the virus. Many experimental hosts are also present in the EPPO region as crops (potato, *Brassica campestris* and *B. oleracea*, *Raphanus sativus*, host Cucurbitaceae, *Chenopodium quinoa*), ornamentals (garden petunia/*Petunia hybrida*, *Nicandra physaloides*) or weeds (such as *Datura stramonium*, *Solanum nigrum*, *Arabidopsis thaliana*).

9.3 Biological and other considerations

As known for some other tobamoviruses, ToMMV can also most likely persist until the following crop in plant debris and overwinter in soil. Volunteer tomato seedlings are likely to carry-over into the next crop. These two factors increase the likelihood of establishment. ToMMV may also survive outside of living host plants (see section 2.3).

ToMMV is easily mechanically transmitted. However, as there is much less handling in tomato and pepper crops grown outdoors compared to crops indoors, it is less likely that one infected plant in a field will infect many other plants. Even if weeds may serve as reservoir, transfer to host crops is limited because there are no known vectors.

The growing period for tomato and pepper outdoors is shorter than indoors, which will not allow a high inoculum to build up. Infected plants may not show symptoms and thus infections may remain undetected and uncontrolled.

9.4 Overall rating of the likelihood of establishment outdoors

The EWG concluded there is a high likelihood of establishment outdoors where hosts are grown. The likelihood was not assessed 'very high' because the likelihood of spread is not considered to be very high for outdoor crops. Spread in outdoor crops is limited as there is not much hands-on activities associated with crop management and percentages of infected plants may remain low, therefore the virus may not be spread to other crops, which decreases the likelihood of establishment. ToMMV has, however, many similarities with ToMV which has a worldwide distribution including countries in the EPPO-region.

The uncertainty is moderate: not many cases of ToMMV outbreaks are known outdoors; data are missing on cultivation practices in the EPPO region (e.g. if tomato crops are planted in the same field every year); and there is a lack of data of the virus in other host crops.

<i>Rating of the likelihood of establishment outdoors</i>	<i>Very low</i> <input type="checkbox"/>	<i>Low</i> <input type="checkbox"/>	<i>Moderate</i> <input type="checkbox"/>	<i>High</i> X	<i>Very high</i> <input type="checkbox"/>
<i>Rating of uncertainty</i>			<i>Low</i> <input type="checkbox"/>	<i>Moderate</i> X	<i>High</i> <input type="checkbox"/>

10. Likelihood of establishment in protected conditions in the PRA area

This category (‘protected conditions’) includes production technologies such as: glasshouses, screenhouses, tunnels and covered fields. Tomatoes may be produced under active or passive greenhouses (i.e. with or without heating) (Section 9.1). Although there are many types of growing systems for greenhouse tomatoes, the two principal cropping systems are two crops per year and one crop per year (OECD, 2017) without rotation.

Details on crops in protected conditions in the EPPO region are given in section 9.1 (together with outdoors crops). At least in part of the EPPO region, tomato cultivars carrying the *Tm-2²* resistance gene are likely widely used in greenhouse crops, as well as *Capsicum* carrying *L* genes/alleles (see section 2.7 and 9.2). ToMMV has been found in greenhouses in, for example, Mexico (Li *et al.*, 2013), Tibet (Li *et al.*, 2014 – *Capsicum*), New York (Padmanabhan *et al.*, 2015), Florida (Fillmer *et al.*, 2014), California and South Carolina (Sui *et al.*, 2017a) and under insect-proof shade houses in Mauritius (Maudarbaccus *et al.*, 2021). There have been isolated findings in protected conditions in the EPPO region in Spain (in a research facility) and in Israel (single finding in a seed crop in 2014) (see section 6), but ToMMV is not known to be established in protected conditions in the EPPO region to date (2022-02). To date no outbreak of ToMMV has been reported in greenhouse fruit production in the EPPO region.

Given that host plants of ToMMV can be grown in all EPPO countries in protected conditions, the whole PRA area is considered as having an environment suitable for ToMMV. However, mitigation measures in place for other tobamoviruses, especially ToBRFV, would likely have effect on ToMMV, but it is not known how strictly these measures are applied, and whether all EPPO countries apply such measures. Finally, if the regulatory status of ToBRFV changes, it is uncertain if such practices will be maintained by all countries / growers.

When host plants are grown under protected conditions, conditions are favourable to the development of the crops and therefore to the virus. Since tomato and pepper are crops where crop-handling procedures are very intensive there is a high risk of mechanical spread. No information was sought on crop handling procedures in other hosts grown indoors.

If new growing media is used for each growing period and if strict sanitation measures are applied between crops, the likelihood of establishment would be lower. The used media may be recycled (Diara *et al.*, 2012) in conditions that likely allow the survival of ToMMV.

The EWG concluded there is a moderate likelihood of establishment in protected conditions –this rating takes into account the use of resistant cultivars, although possibly not throughout the region and that, as far as known, establishment has not happened to date.

The uncertainty is moderate (as it is not known whether *Tm-2²* tomato cultivars are used in protected conditions throughout the region; conditions under which *Tm-2²* tomato cultivars may become infected; what resistance level of *Capsicum* cultivars carrying *L1*, *L3* and *L4* have and; whether ToMMV could establish on pea and common bean when grown indoors).

<i>Rating of the likelihood of establishment in protected conditions</i>	<i>Very low</i> <input type="checkbox"/>	<i>Low</i> <input type="checkbox"/>	<i>Moderate</i> X	<i>High</i> <input type="checkbox"/>	<i>Very high</i> <input type="checkbox"/>
<i>Rating of uncertainty</i>			<i>Low</i> <input type="checkbox"/>	<i>Moderate</i> X	<i>High</i> <input type="checkbox"/>

11. Spread in the PRA area

Natural spread

Most likely, natural dispersal (e.g. with water, pollinating insects and birds) of ToMMV will generally remain within the same production area or even within the same production location.

Human-assisted spread

There is a higher likelihood of outbreaks in open field production, especially among tomato cultivars not carrying the *Tm-2²* resistance gene and *Capsicum* cultivars not carrying *L* genes/alleles, but there would be less transmission by mechanical means (except for fresh fruit or seeds). In greenhouse crops, outbreaks are less likely to occur, but there would be mechanical transmission due to high levels of hands-on activities.

Locally, spread will mainly be linked to human-assisted mechanical transmission of ToMMV by workers, visitors, common cultural practices causing wounds or microlesions (i.e: tools and equipment) (Section 2.2 and section 8.1.6). This is considered as one of the reasons for the rapid spread of ToBRFV in Israel, in addition to infected plants for planting (EPPO, 2020b).

For processing tomatoes, the same harvester can be used between different locations and different owners, facilitating the spread of ToBRFV through soil and plant debris. However, tobamoviruses, and their spread, are normally not a problem in field processing tomato production, because of limited hands-on activities and because there is not continual harvesting. *Capsicum* may also be grown for processing (see section 9), no details on cropping practices were sought.

Non-professional tomato and pepper growers may use self-made compost to grow tomatoes and peppers, which may be a source of infection if infected plants and fruits were composted. Inactivation of tobamoviruses during composting have been shown in several studies (Avgelis & Manios, 1989; Suárez-Estrella et al, 2002; Ryckeboer *et al.*, 2002b; Noble & Roberts, 2004 ; Aguilar *et al.*, 2010). However, composting conditions in self-made composting piles may not be sufficient for inactivation.

At longer distances, the pest could be spread with seeds, young plants and fruits (Section 8). There is a large trade of seeds and young plants between EPPO countries.

Tobamoviruses generally spread rapidly. Preliminary data from the Dutch seed collection suggest that ToMMV may have been present for many years already (see section 6. Distribution). If this is the case, spread has not really been observed, possibly because of the wide use of resistant cultivars, and because outbreaks may have been thought to be caused by ToMV.

Uncertainty: possibly overlooked, specific test not widely available until recently and these tests may still not be widely used.

<i>Rating of the magnitude of spread</i>	<i>Very low</i> <input type="checkbox"/>	<i>Low</i> <input type="checkbox"/>	<i>Moderate</i> X	<i>High</i> <input type="checkbox"/>	<i>Very high</i> <input type="checkbox"/>
<i>Rating of uncertainty</i>			<i>Low</i> <input type="checkbox"/>	<i>Moderate</i> <input type="checkbox"/>	<i>High</i> X

12. Impact in the current area of distribution and areas where outbreaks have previously been detected

Little data are available on impacts of ToMMV. Most information is from China (see below). In China, ToMMV has been found in tomato, *Capsicum*, pea and tobacco. ToMMV may become epidemic according to Li *et al.* (2020), who do not, however, present data on yield losses caused by ToMMV nor data showing an increase in the prevalence of the virus in China. In other countries where it has been reported, there is even less information (including on whether it is established, and present in seed production or commercial production). This section includes all information on impact found in the literature, including from countries where the pest is not established. According to the literature, the prevalence of ToMMV in crops and within

countries appears to be low (see examples below). Environmental or social impacts have not been reported to date.

The impacts of ToMMV may not be fully known because outbreaks could have been thought to be ToMV, and therefore not reported.

On tomato

In Hainan (China), an outbreak on tomato crops in greenhouses and fields in 2016 is described as a ‘devastating disease which seriously reduced production’, with symptoms developing ‘very quickly’ (Zhan *et al.*, 2018). However, no details are provided that clarify/quantify these statements.

In a survey in 13 provinces of China in 2013-2017, ToMMV infection in tomato was detected in four provinces (Yunnan, Hainan, Liaoning and Inner Mongolia), in 18 out of 520 samples from symptomatic plants in total (Li *et al.*, 2020). The article does not provide information on disease incidence. Li *et al.* (2020) also mentions that ToMMV is likely to become one of the most harmful viruses on vegetable crop production, especially in Solanaceae crops in the future. However, this statement does not seem to be supported by data available to date.

In the Mauritius outbreak, symptoms were observed on about 10% of plants in a shade house (*Tm-2²* cultivar; infection possibly linked to high temperature), and 75-80% in two others (one susceptible cultivar and one *Tm-2²* cultivar) (Maudarbaccus *et al.*, 2021).

In the isolated finding in Israel in 2014 (the virus has not been found since – see section 6), 20% of tomato plants (plants without resistance gene *Tm-1*, *Tm-2* or *Tm-2²*) in a seed-production greenhouse showed severe mosaic symptoms and fern-like leaf deformations that reduced production from infected plants (Turina *et al.*, 2016; B. Geraats, pers. comm., 2021-12).

In Brazil, when ToMMV was identified in São Paulo, no survey on incidence and importance was carried out. Based on personal experience, tobamoviruses are not frequently observed on tomato and are not causing relevant damage to the tomato crop in Brazil (A. Nagata, Embrapa, Brazil).

In the Czech Republic, positive test results for ToMMV had a negative impact on operators only if they wanted to export seed to third countries, where ToMMV was regulated (NPPO of the Czech Republic; J. Bustová, pers. comm., 2022-01).

On Capsicum

In a survey in 13 provinces of China in 2013-2017, ToMMV was found in *Capsicum* in five provinces (Yunnan, Hunan, Hainan, Shaanxi and Tibet), in 24 out of 958 samples from symptomatic plants in total (Li *et al.*, 2020). The article does not provide information on disease incidence.

In a study in five provinces of China in 2013-2015, Li *et al.* (2017) detected ToMMV in *Capsicum* samples from symptomatic plants from Yunnan, Tibet and Hunan and report an “overall incidence of naturally infected pepper” of 3.56%. However, it is not clear from the paper if this is the percentage of samples that tested positive, or the percentage of infected plants in the field in these provinces. It is possibly a percentage of positive samples over the total number of samples collected in these provinces (as in Li *et al.*, 2020).

On eggplant

Eggplant is an uncertain host (see section 7), but mixed infections of ToMMV with TMGMV were detected in symptomatic eggplant plants in China (Chai *et al.*, 2018 & 2019). ToMMV was found in 20 out of 23 samples and TMGMV in each of the 23 samples. Symptomatic plants were observed from 2015 to 2017 and visual observations estimated the symptom incidence to be 20-40% (Chai *et al.* 2018). The authors assume that infection with both viruses leads to yield losses. However, to date, the role of ToMMV in the disease is not confirmed.

On pea, common bean, tobacco

No information was found. In addition, for tobacco, it is not clear if ToMMV was found in single infections or only in mixed infections.

Control measures

No evidence was found in the literature on ToMMV that control measures are applied. However, control would rely on typical measures against tobamoviruses, such as:

- use of resistant cultivars.
- use of virus-free seeds and planting material (Luria *et al.*, 2017).
- seed treatment
- treatment of water
- crop inspection
- good practices for workers
- sanitation measures, including disinfection of tools.
- avoidance of fields where the previous crop was infested.

The EWG concluded that ToMMV appears to have caused little impact to date, and believed that, if impact had occurred, it would have been reported. In particular in China, ToMMV is present in many provinces, but no major impact is documented in the publications available, which tested limited numbers of samples.

A high level of infestation was reported in one case (Mauritius outbreak), and ToMMV is also known to have impact on trade for countries exporting seeds. Because of this, the EWG rated impact as moderate.

The uncertainty is moderate (lack of data from countries where ToMMV occurs (including for tomato and *Capsicum*, but also pea, common bean and tobacco); possible under-reporting (including cases where outbreaks thought to be ToMV may have been caused by ToMMV); no known data supporting the strong wordings in Chinese articles e.g. ‘devastating’, ‘epidemic’)

<i>Rating of the magnitude of impact in the current area of distribution</i>	<i>Very low</i> <input type="checkbox"/>	<i>Low</i> <input type="checkbox"/>	<i>Moderate</i> X	<i>High</i> <input type="checkbox"/>	<i>Very high</i> <input type="checkbox"/>
<i>Rating of uncertainty</i>			<i>Low</i> <input type="checkbox"/>	<i>Moderate</i> X	<i>High</i> <input type="checkbox"/>

13. Potential impact in the EPPO region

There are little data on the impact of ToMMV where it occurs, and the assessment of the potential impact is based on what data there is and comparison with related viruses, with some caveats and uncertainties.

The PRA on ToBRFV (EPPO, 2020b) discusses extensively the potential economic impacts that may occur for such a tobamovirus on tomato and *Capsicum*. Similarly, pea and common bean are also important crops in the EPPO region, and tobacco in part of the region.

ToMMV may have impact on export of host commodities to countries where the pest is regulated (as has happened in the Czech Republic). The few figures available in section 12 show that ToMMV may have some impact on crops, at least on tomato. Chitambar (2015) notes that losses similar to those caused by the closely related ToMV can be expected, i.e. yield losses up to 25% in infected non-resistant greenhouse or field-grown susceptible tomato crops (citing CABI, 2015). This is overall not reflected in the data available to date, but there is one case of high incidence in two shade houses in Mauritius.

Fresh fruit production (in protected crops or in the field) is more likely to be affected, as well as hybrid seed production locations in the field. Tobamoviruses (such as ToBRFV) do not generally cause major problems in field processing tomato and *Capsicum* production, because of limited hands-on activities in these crops.

Overall, the potential impact of ToMMV in the PRA area will very much depend on the susceptibility of cultivars that are being grown in EPPO countries:

- Tomato cultivars carrying *Tm-2²* appear to be generally resistant and available evidence also indicate that *Capsicum* cultivars carrying *L*-genes may be resistant. The EWG noted that this is probably the reason that no outbreaks in fruit crops have been reported thus far in Europe.

The Dutch NPPO (2020) notes that “it is estimated by Dutch breeding companies that nearly 100% of the tomato and pepper cultivars grown in the Netherlands (for the fresh market) are resistant to ToMMV and they do not consider ToMMV as an emerging threat (Information from Plantum, Dutch association for the plant reproduction material sector, <https://plantum.nl/>, September 2020). One Dutch company stated that all their indeterminate tomato cultivars have ToMMV resistance through *Tm-2²* (Plantum, 2021-12).

In the UK, tomato varieties available have “high resistance” to ToMV which could convey resistance to ToMMV (UK PRA, 2021).

In Spain, García-Arenal & McDonald (2003, citing the *Vademecum de variedades horticolas*. Portagrano, Almería, Spain; Marín-Rodríguez, J. 2000) mention that about 80% of fresh market cultivars carried the *Tm-2²* gene (at that time).

In Turkey, Kabas *et al.* (2021), in a study on the presence of *Tm-2²* and *Tm-1* in *Solanum* accessions, found that amongst three cultivated tomato varieties, one local variety (Lice) and a commercial hybrid (Torry F1 - Syngenta) carried *Tm-2²*, while a second local variety (Ayaş) did not. Lice and Ayas are grown on limited areas in open field, while Torry is grown over a very large area, especially in greenhouse production (estimated 40000 seed pockets/year) (A. Kabas, pers. comm., 2022-02). From information available from Turkey (E. Ince, pers. comm.), it appears that almost all cultivars from Turkish seed companies carry the *Tm-2²* gene, and that this gene is present in most varieties in catalogues of local companies or registered in the Variety Registration and Seed Certification System (TTSM).

The EWG noted that, in the EPPO region, *Tm-2²* would at least be widely used in greenhouses, which limits the potential impact. There was an uncertainty on whether *Tm-2²* is used for fresh fruit production in the field, and *Tm-2²* cultivars are probably not used for processing tomato production. In addition, there may be EPPO countries where it is less common to use *Tm-2²* cultivars, even in greenhouse production.

At higher temperatures, which may be attained in greenhouse production in some countries of the EPPO region, or in a context of climate change, there may be a break-down of resistance and some impact may occur (see sections 2.7 and 12).

Impact on *Tm-2²* cultivars may occur in other circumstances, potential cause as yet unknown, as shown in Mauritius with 75-80% infection on one cultivar in a shade house; however, this is the only case known to date.

- ToMMV has not so far spread or caused outbreaks to the same extent as ToBRFV. Nevertheless, ToMMV seems to have a broader host range than ToBRFV, and may have the potential to spread to other hosts than those identified to date.
- Seeds may be treated against ToMV during the production process (see Annex 4, Point 5). If these treatments are also effective against ToMMV, this would reduce the impact of ToMMV. However, the efficacy of such treatments is not confirmed to date.
- There have been fewer reports of outbreaks and yield losses to date for ToMMV compared to ToBRFV, and none in EPPO countries. Based on currently available information ToMMV seems to present less of a threat than ToBRFV (which has spread rapidly and causes damage), however, some sources hypothesise that it may become an important virus (see section 12).
- There is no information to date on the impact of ToMMV on pea, common beans or tobacco, and little information for *Capsicum*. *L1*, *L3* and *L4* genes/alleles seem to be effective against ToMMV in *Capsicum* but the EWG did not find scientific studies that confirm their efficacy. There is uncertainty how common *L1*, *L3* and *L4* -cultivars are used in the entire EPPO-region.
- The potential impact is higher in fruit crops produced for the fresh market than in fruit production for processing. In the latter, tobamoviruses spread slower because of less crop handling activities. On the other hand, cultivars used for producing fruit for processing are less likely to carry resistance genes.
- In the PRA for ToBRFV (EPPO, 2020), there was a concern that **mixed infections with established viruses (e.g. in tomato with TMV, ToMV, TYLCV, PepMV) might occur with a risk of synergism, or even recombination events, which might lead to new viral disease problems in the future (as noted by Hansen *et al.*, 2010 for emerging viruses)**. For example a first infection of host plants with another virus may allow a second infection with ToMMV usually blocked by resistance genes/alleles or, conversely, a first infection of plants with ToMMV may allow a second infection with another virus usually blocked by resistance genes/alleles (such as ToBRFV). There is no data on the consequences of mixed infections with ToMMV to date (see recommendation in section 18).

Environmental impacts are not expected. Social impacts may occur if ToMMV decreases the quality of fruits and cause shortages (UK PRA, 2021). This has, however, not been reported from countries where the virus occurs.

The EWG concluded that there is no strong evidence that potential impact will be much different than in the current area of distribution. Fresh fruit production (in protected crops or in the field) is more likely to be affected, as well as hybrid seed production locations in the field. However, impact on tomato and *Capsicum* will depend mostly on the susceptibility of cultivars that are being grown in EPPO countries. Resistant cultivars (carrying the *Tm-2²* gene for tomato and *L* genes/alleles for *Capsicum*) may not be used in all types of crops and throughout the EPPO region. The direct impact (yield losses, additional crop protection costs) may be low in areas where nearly all tomato crops carry the *Tm-2²* resistance gene and not different from that of ToMV. However, while a resistance break has not been shown to date, with the exception of one reported case of high infection rate of a tomato *Tm-2²* cultivar, there is some evidence that high temperatures may help to cause expression of symptoms in some *Tm-2²* cultivars. The uncertainty is moderate (similar uncertainties as in section 12).

<i>Rating of the magnitude of potential impact in the PRA area</i>	<i>Very low</i> <input type="checkbox"/>	<i>Low</i> <input type="checkbox"/>	<i>Moderate</i> X	<i>High</i> <input type="checkbox"/>	<i>Very high</i> <input type="checkbox"/>
<i>Rating of uncertainty</i>			<i>Low</i> <input type="checkbox"/>	<i>Moderate</i> X	<i>High</i> <input type="checkbox"/>

14. Identification of the endangered area

Potential area of establishment. ToMMV could establish in the whole EPPO region wherever hosts are grown, i.e. throughout the EPPO region in protected conditions, and outdoors south of ca. 63°N.

Endangered area. The evidence suggests that economic impact is most likely on tomato, but with more impact where cultivars that do not carry the *Tm-2²* resistance gene are used. There may also be economic impact on *Capsicum*, wherever grown, but impact may be limited to cultivars that do not carry the *L* resistance genes/alleles. There are uncertainties about the potential impact (see section 13), particularly linked to the effectiveness of the *Tm-2²* and *L* genes/alleles and the extent to which tomato and *Capsicum* cultivars are used with these genes/alleles in the EPPO-region.

15. Overall assessment of risk

Summary of ratings:

	likelihood	uncertainty
Entry (overall)		
Seeds of tomato	high	moderate
Seeds of <i>Capsicum</i>	moderate	high
Plants for planting of tomato (except seeds and pollen)	moderate	moderate
Plants for planting of <i>Capsicum</i> (except seeds and pollen)	moderate	high
Fruits of tomato and <i>Capsicum</i>	low	moderate
Mechanical transmission (from containers, tools, equipment and conveyance vehicles associated with the hosts production and supply chain)	low	high
Establishment outdoors	high	moderate
Establishment in protected conditions	moderate	moderate
Spread	moderate	high
Magnitude of impact in the current area of distribution and areas where outbreaks have previously been detected	moderate	moderate
Magnitude of potential impact in the PRA area	moderate	moderate

A few factors affected different stages of the assessment:

- The use of resistant cultivars (*throughout this section, 'resistant cultivars' means carrying the Tm-2² resistance gene for tomato, and L genes/alleles (L1, L3 or L4) for Capsicum*) is considered to reduce ToMMV occurrence, infection and damage. Due to the risk posed by tobamoviruses in crops with high levels of hands-on management, resistant cultivars are widely used especially in intensive commercial fresh fruit production of tomato and *Capsicum* in protected conditions (at least in the EU). This may also be the case in outdoor crops for fresh fruit production, but not for tomato cultivars for processing grown in the field. However, information is not available for all EPPO countries.
- ToMMV may have a wider distribution than currently reported, including in EPPO countries. It may have arrived already many times on imported seeds. ToMMV-specific tests have not been commonly used in the past, and outbreaks of ToMMV could have been thought to be ToMV, and therefore not reported.
- Although pea, common bean and tobacco are hosts, it was not possible to cover them in several steps of the PRA due to lack of information.

Entry: The likelihood of entry was considered as high with a moderate uncertainty, the highest ratings being seeds of tomato and *Capsicum*, and plants for planting (except seeds and pollen) of tomato and *Capsicum*. ToMMV has been found to be associated with seeds. It is noted that transfer is less likely if the cultivar carries the resistance genes above. However a small portion of the plants may still become infected. The likelihood of entry on fruit of tomato and *Capsicum*, and on containers, tools, equipment and conveyance vehicles associated with the hosts production and supply chain, was considered as low. The uncertainty associated to all pathways is moderate to high.

Establishment: The likelihood of establishment of ToMMV was rated high for outdoors in the EPPO region where host plants are grown (with a moderate uncertainty). Under protected conditions, the likelihood of establishment was assessed to be moderate (with a moderate uncertainty), because of the wide use of resistant cultivars, and because establishment in protected conditions does not appear to have happened to date.

The magnitude of spread was rated moderate with a high uncertainty. The pest could spread locally by natural dispersal (e.g. with water, pollinating insects and birds) within a production area or more widely via human-assisted mechanical transmission by workers, visitors, tools and equipment (including plastic containers used for the transport of fresh tomatoes) as well as with the trade of plants for planting, seed and fruit.

Impact (economic and social) was rated moderate with a moderate uncertainty. Fresh fruit production (in protected crops or in the field) is more likely to be affected, as well as sites producing hybrid seed in the field. However, impact on tomato and *Capsicum* will depend mostly on the susceptibility of cultivars that are being grown in EPPO countries. Resistant cultivars may not be used in all types of crops and throughout EPPO countries. The direct impact (yield losses, additional crop protection costs) may be low in areas where nearly all tomato crops carry the *Tm-2²* resistance gene and not different from that of ToMV. However, while a real resistance break has not been shown to date, in one case, ToMMV was reported to be the causal agent for a disease outbreak on tomato cultivars with the *Tm-2²* gene, and there is some evidence that high temperatures may help to cause expression of symptoms in some *Tm-2²* cultivars.

The EWG noted that information is lacking regarding the current situation of ToMMV in the EPPO region. (Preliminary) tests of tomato and *Capsicum* seed batches from the Dutch seed bank detected ToMMV on a large number of batches, while it was not found in field samples in the Netherlands. ToMMV was found in three seed crops in the Czech Republic, but not in the Czech seedbank, and it was not found in the field or seed samples in several other EPPO countries (see section 6). The EWG recommended that ToMMV-specific testing is performed on field samples and seed banks to verify the situation. This recommendation and others are detailed in section 18.

There was no agreement in the EWG regarding whether ToMMV should be recommended for regulation.

In favour of recommending for regulation	Not in favour of recommending for regulation
Seven members believed that regulating ToMMV as a quarantine pest would allow preventing its spread and possible impact.	Two members were not in favour of recommending the virus for regulation as a quarantine pest until more information regarding the uncertainties is available.

<p>If ToMMV is not regulated now, it is likely to spread in particular with the seed trade, and it will then be too late to stop its spread. The members outlined that there is uncertainty on potential impact, but there is one report of concern with a high level of infestation in Mauritius on a tomato cultivar carrying the <i>Tm-2²</i> gene.</p> <p>In conclusion, they suggested that ToMMV should be recommended for regulation, for the time being, with phytosanitary measures for seeds and plants for planting of tomato and <i>Capsicum</i>.</p>	<p>ToMMV was first described in 2013 in Mexico but data indicate that the virus had already been around for many years. ToMMV was found in a collection from 1992 in Brazil and preliminary data from the Dutch gene bank suggest that the virus has been present in the EPPO-region before 2013. In recent years, many findings of ToMMV have been reported on tomato and <i>Capsicum</i> seeds (see section 8.1.1) but it may have been associated with seeds long before. ToMMV-specific tests have not been commonly used in the past, and findings of ToMMV could have been thought to be ToMV, and not reported or reported as ToMV. Thus, ToMMV may have been (locally) present in the EPPO region for some time and has been mistaken as ToMV. Since its description, some findings have been reported in Israel (one finding in a tomato glasshouse of a seed producer in 2014, but not found since), Spain (one detection in tomato in a research glasshouse in 2015) and Czech Republic (in two asymptomatic tomato seed crops and one asymptomatic <i>Capsicum</i> seed crop in 2020). It is not known (or not reported) how the virus was introduced at those places.</p> <p>Evidence available indicate that effects of ToMV and ToMMV on tomato and <i>Capsicum</i> are not much different. Experimental studies indicate that the <i>Tm-2²</i> gene in tomato is similarly effective against ToMV and ToMMV and <i>L</i> genes/alleles (<i>L1</i>, <i>L3</i> and <i>L4</i>) in <i>Capsicum</i> also seem to be effective against ToMMV.</p> <p>Thus, ToMMV and ToMV seem very similar. ToMV is considered widely distributed and is not regulated in most of the EPPO region. ToMMV and ToMV may have long been thought to be the same species (ToMV). Phytosanitary measures against ToMMV alone are, therefore, not recommended unless evidence indicates that ToMMV is clearly a bigger threat than ToMV.</p>
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The PPM decided to not recommend the addition of ToMMV to the List of pests recommended for regulation for the time being, and therefore to not propose risk management options. However, it stressed the importance of gathering more information and took note of possible topics for research in section 18. The Panel commented that more guidance for testing would be valuable and recommended that additional surveillance is performed in the EPPO region.

Stage 3. Pest risk management

16. Phytosanitary measures

The EWG reviewed phytosanitary measures that could be recommended if ToMMV is recommended for regulation and restricted these measures to the pathways ‘seeds’ and ‘plants for planting (except seeds and pollen)’ of tomato and *Capsicum*. The measures were similar to those for ToBRFV (EPPO, 2020b), and the EWG added considerations specifically related to ToMMV. Because the PPM did not recommend the addition

of ToMMV to the List of pests recommended for regulation for the time being, the phytosanitary measures and additional considerations are not presented in this PRA, but are available at : <https://upload.eppo.int/download/1414o49dba66de>

Similarly, eradication and containment are not covered in this PRA, but details on measures to be applied in the case of an outbreak indoors, or in the open field are developed in the EPPO PRA for ToBRFV (EPPO, 2020b).

17. Uncertainty

The main uncertainties in this PRA relate to:

- seed-borne transmission of ToMMV
- lack of data on pea, common bean and tobacco
- host status of eggplant
- lack of data on the presence of ToMMV in the EPPO region and worldwide
- lack of information about its presence in seed banks/seed companies
- impact of ToMMV on *Tm-2²* / *L* cultivars in its current area of distribution – lack of clarity on reasons why some resistant cultivars have been affected by ToMMV in some situations.
- extend of use of cultivars with *Tm-2²* / *L* resistance genes in EPPO countries
- whether seed treatments commonly used during seed production against ToMV are effective against ToMMV.
- lack of trade data for some EPPO countries.

18. Remarks

The EWG made the following recommendations to NPPOs and research institutes in EPPO countries:

- to perform ToMMV-specific tests on field samples and in seed banks to verify the situation of ToMMV. In particular:
 - Surveys using test methods that can differentiate ToMMV from ToMV in countries producing tomato and *Capsicum* seeds, fruits and plants, possibly also other hosts (pea, common bean and tobacco).
 - Test of accessions in seed gene banks, as done in the Netherlands (see section 6).
 - Retesting historic samples of tobamoviruses (e.g. in collections, back-up samples) to verify the identity of viruses (see section 2.8 and 6).
- to validate test protocols. This is ongoing within an Euphresco project [Validation of molecular diagnostic methods for the detection and identification of tomato mottle mosaic virus (ToMMV) (2022-A-394)] and interested laboratories are invited to liaise with their Euphresco representative to join this project.
- to fully investigate findings of tobamovirus symptoms using methods that distinguish ToMV, ToMMV and ToBRFV, and not only trying to ascertain if ToBRFV is present.
- to test imported commodities, especially tomato and *Capsicum* seeds. This can provide useful information about the current distribution of ToMMV, provided the origin of the seeds is fully documented.
- to perform further studies on the efficacy of seed treatments, including those commonly used against ToMV
- to improve the traceability of the seeds and transparency of their origin.
- in situations that favour tobamoviruses (see section 2.7), to promote the use of tomato *Tm-2²* cultivars and *Capsicum L* cultivars to reduce the risk of infection by ToMMV (see section 2.7).
- to carry out more research on the symptoms and impacts of mixed infections with other viruses, [including the possible role of a first infection with another virus in allowing a second infection with ToMMV \(e.g. to clarify the situation on tobacco and eggplant in China, as well as their host status and pathogenicity\), or of a first infection by ToMMV to allow a second infection with another virus usually blocked by resistance genes/alleles \(or the reverse\).](#)
- to perform studies on the survival of the virus in soil and in water used for irrigation, as well as on different surfaces (including treatment methods and composting).

19. References (including for Annexes)

(all links were accessed in December 2021, except where a specific date is indicated) [Blue: reference is cited in text extracted from the PRA on ToBRFV]

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ANNEX 1. Isolates deposited in Genbank as ToMV and corresponding to ToMMV

Isolates in Table 1 below were deposited in Genbank as ToMV and are considered to correspond to ToMMV (in references mentioned in the last column). Some records extend the host range and the distribution of ToMMV (reflected in sections 6 & 7). These isolates are still under the name ToMV in Genbank, and this PRA clearly mentions when information relates to them.

Table 1. Isolates deposited in Genbank as ToMV but considered in the literature to be ToMMV

Isolate	Country	Collected from	Collection date or article submission date*	Reported as ToMMV by
AF411922#	Brazil (Sao Paulo)	Tomato	2001*	Li <i>et al.</i> , 2017, 2020; Turina <i>et al.</i> , 2016
AM411425#	Brazil (Sao Paulo)	<i>Capsicum</i> spp.	2006* (unpublished article)	Li <i>et al.</i> , 2017, 2020
AM411430#	Brazil (Sao Paulo)	<i>Capsicum</i> spp.	2006* (unpublished article)	Li <i>et al.</i> , 2017, 2020
AM411431	Brazil (Sao Paulo)	<i>Capsicum annuum</i>	2006 (unpublished article)	Alavi & Massumi, 2014 (as ToMV, see below table)
KT222999	Brazil (Sao Paulo)	tomato	1992	Nagai <i>et al.</i> , 2018
JX025564	China	tomato	2012	Li <i>et al.</i> , 2017, 2020
JX112024#	Iran	Tomato	2008	Li <i>et al.</i> , 2017, 2020
JX112025#	Iran	Tomato	2007	Li <i>et al.</i> , 2017, 2020
JX121575#	Iran	Tomato	2007	Li <i>et al.</i> , 2017, 2020
JX121576#	Iran	Tomato	2007	Li <i>et al.</i> , 2017, 2020
JX121574#	Iran	<i>Capsicum</i> spp.	2007	Li <i>et al.</i> , 2017, 2020
JX121570#	Iran	<i>Phaseolus vulgaris</i> cv. Red Kidney (‘kidney bean’) [kera 2]	2008	Li <i>et al.</i> , 2017, 2020, Alavi & Massumi, 2014
HQ593616	Iran	<i>Chenopodium murale</i>	2009	Li <i>et al.</i> , 2017, 2020

The JX isolates from Iran were part of a comprehensive study on Iranian isolates of ToMV (Alavi & Massumi, 2014). Samples were collected in the field on tomato, pepper, beans and weeds, and isolates of ToMV extracted. Molecular analysis (based on PCR; CP gene) was performed on the Iranian isolates and on some worldwide isolates registered as ToMV in Genbank. Isolates separated into three groups. Group III was composed only of the JX Iranian isolates above and the first four Brazilian isolates above. Three of the four Brazilian isolates are mentioned as being ToMMV by other authors (Table 1). The fourth one, AM411431, grouped with others in Alavi & Massumi (2014) and was added to Table 1.

It should be noted that the Brazilian isolates above, which are now considered to be ToMMV, have also been used in genetic studies on ToMV (i.e. with the assumption that they were isolates of ToMV). For example, Rangel Aranguren (2014), using many isolates of ToMV, obtained a clear clade separation of the Brazilian isolates above (forming clades II and III) from all other ToMV isolates (forming clade I). No other case of allocation of new isolates to ToMV based on their homology with the isolate AF411922 known to be ToMMV were found in the literature. A NCBI blast analysis with all isolates in the table above with check of the highest similarity (all hits with more than 90% similarity), showed that no other isolates of ToMMV has been uploaded to NCBI as ToMV (as of 1 February 2022).

ANNEX 2. Symptoms of ToMMV



Fig. 1. Mottle mosaic symptoms on tomato plants without *Tm-2²* gene infected by ToMMV in experiments (from Sui *et al.*, 2017a)



Fig. 2 Brown necrotic lesions expressed on leaves and fruits of a tomato plant with *Tm-2²* gene infected by ToMMV in experiments (from Sui *et al.*, 2017a)

ANNEX 3. Symptoms on experimental hosts

Remarks:

- Sui *et al.* (2017a) indicate separately symptoms on inoculated and non-inoculated leaves. Only symptoms on non-inoculated leaves (i.e. systemic) are given below.
- Host marked with *: experimental hosts listed in Li *et al.* (2020) are considered uncertain (see section 7). Nevertheless, it is noted that symptoms described on some of these plants seem to correspond to systemic reaction.

Experimental host	Symptoms (reference)
Solanaceae	
<i>Datura stramonium</i>	<ul style="list-style-type: none"> • Necrosis, plant death (Sui <i>et al.</i>, 2017a)
<i>Nicandra physaloides</i> *	<ul style="list-style-type: none"> • Mosaic (Li <i>et al.</i>, 2020) • Mottle, blistering, distortion (Li <i>et al.</i>, 2017 with photo)
<i>Nicotiana benthamiana</i>	<ul style="list-style-type: none"> • Mosaic, distortion, plant death (Sui <i>et al.</i>, 2017a) • Chlorosis, yellowing (Li <i>et al.</i>, 2020) • Chlorosis (Li <i>et al.</i>, 2017 with photo) • Severe stunting, systemic crinkling, leaf distortion with apical necrosis (Ambrós <i>et al.</i>, 2017 with photo) • Leaf chlorosis and deformation (Webster <i>et al.</i>, 2014) • Crinkling and yellowing on upper leaves, later serious necrosis of apical shoot (Zhan <i>et al.</i>, 2018)
<i>Nicotiana glutinosa</i> *	<ul style="list-style-type: none"> • Necrotic spot (Li <i>et al.</i>, 2020)
<i>Nicotiana rustica</i>	<ul style="list-style-type: none"> • Mosaic, plant death (Sui <i>et al.</i>, 2017a) • Blistering, distortion, necrosis (Li <i>et al.</i>, 2020; Li <i>et al.</i>, 2017 with photo)
<i>Petunia hybrida</i>	<ul style="list-style-type: none"> • Blistering, distortion (Li <i>et al.</i>, 2020) • Mosaic, distortion and leaf narrowing (Li <i>et al.</i>, 2017 with photo) • Asymptomatic (on inoculated and non-inoculated leaves) but systemic (RT-PCR on non-inoculated leaves) (Sui <i>et al.</i>, 2017a)
<i>Physalis alkekengi</i> *	<ul style="list-style-type: none"> • Chlorosis, mottle (Li <i>et al.</i>, 2020) • Mosaic, chlorosis (Li <i>et al.</i>, 2017 – with photo)
<i>Physalis angulata</i>	<ul style="list-style-type: none"> • Mosaic (Sui <i>et al.</i>, 2017a)
<i>Physalis pubescens</i>	<ul style="list-style-type: none"> • Mosaic (Sui <i>et al.</i>, 2017a)
<i>Solanum melongena</i> * (see unconfirmed hosts in sect. 7)	<ul style="list-style-type: none"> • Mottle, necrosis (Li <i>et al.</i> 2017, 2020)
<i>Solanum nigrum</i>	<ul style="list-style-type: none"> • Yellow mosaic (Sui <i>et al.</i>, 2017a)
<i>Solanum pimpinellifolium</i>	<ul style="list-style-type: none"> • Stunting, leaf narrowing, light veins, mosaic (Nagai, 2017)
<i>Solanum tuberosum</i> *	<ul style="list-style-type: none"> • Mottle, yellowing, necrosis (Li <i>et al.</i>, 2020)
Amaranthaceae	
<i>Chenopodium amaranticolor</i>	<ul style="list-style-type: none"> • Necrotic lesion, chlorotic lesion (isolate AF4111922 in Moreira <i>et al.</i>, 2003. isolate published as ToMV but later mentioned as being ToMMV – see section 7).
<i>Chenopodium quinoa</i>	<ul style="list-style-type: none"> • Chlorotic lesion (Sui <i>et al.</i>, 2017a) • Necrotic local lesions (Ambrós <i>et al.</i>, 2017 with photo)
<i>Gomphrena globosa</i>	<ul style="list-style-type: none"> • Mosaic (Sui <i>et al.</i>, 2017a)
Asteraceae	
<i>Glebionis coronaria</i>	<ul style="list-style-type: none"> • Asymptomatic (on inoculated and non-inoculated leaves) but systemic (RT-PCR on non-inoculated leaves) (Sui <i>et al.</i>, 2017a)
Brassicaceae	
<i>Arabidopsis thaliana</i>	<ul style="list-style-type: none"> • Stunting, leaf curling, systemic crinkling, leaf malformation (Ambrós <i>et al.</i>, 2017 with photos)
<i>Brassica campestris</i> *	<ul style="list-style-type: none"> • Mild mottle (Li <i>et al.</i>, 2020) • Mottle (Li <i>et al.</i>, 2017 with photo)
<i>Brassica chinensis</i> *	<ul style="list-style-type: none"> • Chlorosis, mild mottle (Li <i>et al.</i>, 2020) • Symptoms not described in Li <i>et al.</i> (2017)
<i>Brassica oleracea var. italica</i> *	<ul style="list-style-type: none"> • Asymptomatic but detected by RT-PCR (Li <i>et al.</i>, 2020) • Symptoms not described in Li <i>et al.</i> (2017)
<i>Brassica oleracea var. botrytis</i> *	<ul style="list-style-type: none"> • Asymptomatic but detected by RT-PCR (Li <i>et al.</i>, 2020) • Symptoms not described in Li <i>et al.</i> (2017)
<i>Brassica pekinensis</i> *	<ul style="list-style-type: none"> • Chlorosis, mild mottle (Li <i>et al.</i>, 2020)

Experimental host	Symptoms (reference)
	<ul style="list-style-type: none"> Mottle (Li <i>et al.</i>, 2017 with photo)
<i>Raphanus sativus</i> *	<ul style="list-style-type: none"> Mild mottle (Li <i>et al.</i>, 2020) Symptoms not described in Li <i>et al.</i> (2017)
Cucurbitaceae	
<i>Cucumis sativus</i> *	<ul style="list-style-type: none"> Asymptomatic but detected by RT-PCR (Li <i>et al.</i>, 2020)
<i>Cucurbita moschata</i> *	<ul style="list-style-type: none"> Asymptomatic but detected by RT-PCR (Li <i>et al.</i>, 2020)
<i>Cucurbita pepo</i> *	<ul style="list-style-type: none"> Chlorosis, mild mottle (Li <i>et al.</i>, 2020)
Verbenaceae	
<i>Verbena halei</i> (as <i>Verbena officinalis</i> var. <i>halei</i>)	<ul style="list-style-type: none"> Asymptomatic (on inoculated and non-inoculated leaves) but systemic (RT-PCR on non-inoculated leaves) (Sui <i>et al.</i>, 2017a)

ANNEX 4. Factors affecting association of ToMMV with the seed pathway

Apart from one change under point 5 with regards to seed treatments, this text is from the PRA on ToBRFV (EPPO, 2020b) and also applies to ToMMV.

The association of the pest with the pathway is difficult to assess as the following factors may affect the association of the pest with the seeds:

1 - It is difficult to traceback the geographical origin of seed lots and therefore to be certain of its pest status. Individual consignments of hybrid seeds are often composed of mixed lots of seeds produced in several countries of origin, which increases the probability of association of the virus with the seed and the presence of infected seed in the individual seed lots derived from the bulked seed.

The production of some hybrid tomato seed lots typically involves activities in several countries performed (Figure 2) by major international seed companies (e.g. Monsanto [belongs now to Bayer], Syngenta, Groupe Limagrain, Rijk Zwaan, Bayer CropScience, Advanta, Sakata and East-West Seed) (Bai & Lindhout, 2007; Mordor Intelligence, 2018). This sector is experiencing a high degree of concentration, as 95% of the EU market is in the hands of only five companies, which control 45% of the tomato varieties (Mammana, 2014). Plant lines used to produce hybrid seeds are often grown, selected and multiplied in two or three countries successively (AGDAWR, 2018; ISF, 2017). Larger seed companies typically contract out the production and multiplication processes to farmers, farmers' associations or private firms, often in countries with low production costs (AGDAWR, 2018).

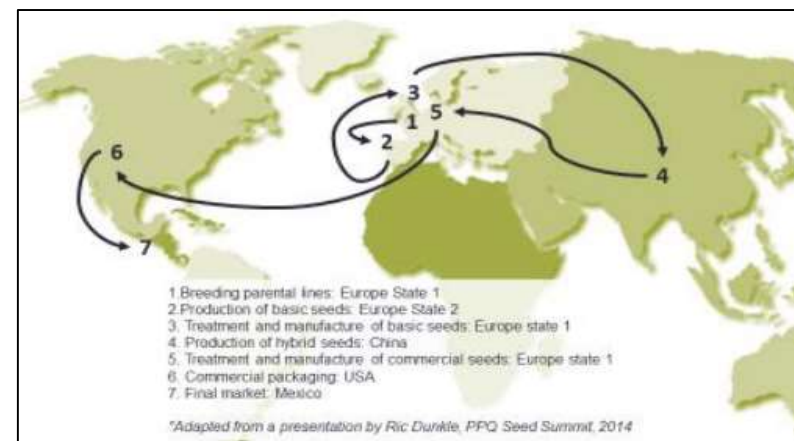


Figure 2. Seeds, a complicated trade model (ISF, 2017)

As an example, two parental lines may be bred in the Netherlands, then larger quantities of seed of these lines (basic seed) may be produced in France or Spain, and this basic seed may then be grown in Thailand or China where the tomato flowers are cross-pollinated to produce the hybrid seed (AGDAWR, 2018; ISF, 2017). In another example, parental lines bred and selected in a country in the Northern Hemisphere were sent to a country in the Southern Hemisphere where breeding and selection continued so that two seasons of breeding were achieved in a single year. During processing and shipment, tomato seed from one lot¹⁶ is commonly divided up into a series of batches. Batches may be handled and packaged in different ways for different end-users. Each time a batch is divided, treated or repackaged, the batch and its derivatives are usually assigned new batch numbers (AGDAWR, 2018). After production, tomato seed batches are transhipped by airfreight via other countries. As an example, some tomato seeds sent to Australia are transhipped through France, Israel, Japan, the Netherlands or the USA (AGDAWR, 2018). Portions of a lot are sold to fruit production growers and nurseries in many countries (AGDAWR, 2018).

The International Seed Federation (ISF) has also recognised that phytosanitary certification of seed can be challenging because the final destination of the seed may not be known when the seed is produced (ISF, 2017). Failing to retain the seed lot number, which distinguishes the place and season of production, and re-packaging and re-labelling of seed lots may also make phytosanitary certification difficult or unreliable.

2 - By growing plant lines in different places, the plant lines may be exposed to a higher likelihood to get infected

In China, the places where seed crops are grown outside change relatively often, as tomato crops for seed production are typically rotated every year in response to pest and pathogen pressure, which may expose them to infection by viruses (EU Commission, 2019; Gould, 1992). Moreover, the location of crops will change as the farm businesses, who are independent from the seed trading businesses, make decisions about subcontracted seed production every year (Venkateswarlu, 2007). However, seed production for the high-tech tomato production in the EU is

¹⁶ a production lot originates in one farm or field and is produced in one season.

largely done following GSPP (Good Seed and Plant Practices, <https://gspp.eu/>) standards, so under insect-proof greenhouses with high hygiene standards. The majority of tomato and pepper seeds imported from Guatemala, Israel, Kenya, Tanzania (countries evaluated during EU commission audits; EU Commission, 2019) are produced in high-tech insect-proof greenhouses.

3 - Combining seeds from different sources into a single consignment may also introduce infected seeds to healthy seed lots.

Combining may introduce infected seeds to healthy commercial lots. Seed lots that include very small numbers of infected seeds may be difficult to detect depending on the number of infected seeds and on the sensitivity of the detection method. However, the commercial lots can be mixed and homogenised to provide a homogeneous germination level. Combining of vegetable production seed lots is a commercial practice (Bello & Bradford, 2016; ISF, 2017) but no report was found that indicated how often combining is done. However, assured traceability is one of the key requirements in GSPP, so the combining of seed lots is unlikely to occur in seed lots for high-tech tomato production following this certification scheme.

4 – The seed production process involves many steps and manipulations which may be a source of mechanical transmission and spread of the virus.

The tomato seed production process begins with plant breeding and involves the production of parent lines which are usually hybridised to produce the seed. Hybridisation involves emasculation of flowers and pollination by hand (Cheema & Dhaliwal 2005). This process has the potential to mechanically transmit and spread ToMMV (Figure **Erreur ! Source du renvoi introuvable.**3). Hybrid seeds are more likely to have been mechanically infected by the virus than non-hybrid seeds.



Figure 3. The emasculation process, involving removal of the staminate cone (courtesy H. Bolcan) (APS, 2014)

5 – The seed production process involves regular cleaning, heat and chemical treatments which may reduce the association of the virus with the seed.

After harvesting the fruit, the seed is extracted and cleaned by separating the seed from the pulp. Typically, the pulp is fermented for several hours, washed with an acidic solution, and then washed with water several times. This washing is efficient to decrease the association with some viruses. This extraction process may be done at the place of production.

Seeds are often treated which will decrease the probability of association with the seed pathway:

- ✓ Heat treatments of dry seeds for 2-4 days at 70°C to 76°C are commonly used to eliminate external and internal viruses, apparently without affecting seed germination (APS, 2014; Dombrovsky & Smith, 2017). The optimal time for seed treatment by thermotherapy using dry heat against another tomato tobamovirus, TMV, is 70°C during 24 h (Silva *et al.*, 2011).
- ✓ Chemical treatments are also commonly used on large scale. The most commonly used treatment in commercial seed production is 10% Trisodium phosphate (Na_3PO_4) which is known to eliminate tobamoviruses such as TMV and ToMV (APS, 2014; Córdoba-Sellés *et al.*, 2007; Dombrovsky & Smith, 2017). Treatment with a 10% solution of trisodium phosphate for at least 15 min is considered to eliminate external viruses, apparently without affecting seed germination (APS, 2014). However, Lapidot (pers. comm., 2019 cited in EPPO, 2020b) showed that 30 min treatment was needed for ToBRFV. Other disinfection methods used in commercial seed include the use of 1-9% hydrochloric acid (HCl), 1-5% calcium hypochlorite ($\text{Ca}(\text{OCl})_2$), 1-3% sodium hypochlorite (NaOCl) and tetramethylthiuram disulphide ($(\text{CH}_3)_2\text{NCSS}_2\text{CSN}(\text{CH}_3)_2$).

However, this treatment is sometimes performed after shipment to a facility in another country (AGDAWR, 2018).

Some treatments are commonly applied against ToMV in some countries during seed production, and may also be effective against ToMMV; however, the EWG noted that their effectiveness on ToMMV has not been demonstrated to date.

6 – Existing field inspections and seed-lots testing for the detection of other pests prior to export reduces the likelihood of association

- Exporting countries commonly visually inspect seed crops to check that certain pests are not present in the crop (AGDAWR, 2018). However, careful visual examination of the crops may not enable an inspector to detect the presence of ToMMV as some varieties are symptomless. Infection can also remain symptomless under certain growing conditions.
- Seed companies often test tomato seed-lots for the presence of tobamoviruses as part of their quality system (De Ruiter, 2019).

ANNEX 5. Trade data for pathways seeds and fruit

Eurostat. Data on trade. in 100 kg. lines/rows with no data were deleted.

Table 1. 12099180 Vegetable seeds for sowing (excl. salad beet or beetroot "Beta vulgaris var. conditiva")

Codes in this table: CZ: Czech Rep.; BR: Brazil; CN: China; MX: Mexico

Export by:	CZ	CZ	CZ	BR	BR	BR	CN	CN	CN	MX	MX	MX
Import by :	2018	2019	2020	2018	2019	2020	2018	2019	2020	2018	2019	2020
Austria	68,09	106,73	473,38						0,01	0,03		
Belgium	0,25	0,46	0,58				1000	500,04	625			
Bulgaria	0,27	79,42	15,72					0,23	642,52			
Cyprus	0,01						0,41	110,03	0,23			
Czechia							99,68	73,95	142,72			
Germany	503,72	317,15	377,84				233,62	336,05	318,66		0,76	0,08
Denmark	0,28	0,73	14,16			0,01	21,23	359,58	463,29			
Estonia							0,01	0,01				
Spain	0,51	1,16	1,41	0,33	0,02	0,02	883,43	367,31	286,14	0,25	0,84	0,01
Finland	0,03		0,06									
France	6,94	5,39	241,83	113,5	275,37	383,22	711,13	1077,85	830,31	7,03	15,84	13,22
UK	10,24	9,55	0,06	0,33	10,2		162,69	255,58	1,97			
Greece	3,79	6,32	4,33				292,65	276,57	273,92	0,15		
Croatia	3,99	6	4,02				0,02	0,01	0,29			
Hungary	69,22	139,28	44,7				28,1	32,93	50,99			
Ireland							0,02	0,03	0			0
Italy	484,38	59,06	53,59	34,04	5,95	2,67	2572,62	2218,53	2465,04	0,13	0,6	
Lithuania	115,35	106,1	173,23				415	155	820			
Luxembourg	0,12	0,31	10,48									
Latvia	7,41	24,13	25,76						0,02			0
Malta			0,01				0,02	0	0,01			
Netherlands	8,56	15,06	10,46	39,75	65,4	27,51	6225,4	6349,74	7000,42	57,39	158,29	60,23
Poland	920,08	45,84	1184,61				221,95	153,67	165,93			
Portugal	741,08	365,4	18,34	0,08		1	10,43	16,39	5,53			
Romania	51,61	138,7	36,5				2482,47	2689,82	6090,6			
Sweden		1,95	0,2				0,25	0	0,06			
Slovenia	7,24	3,27	0,3				0		0,49			
Slovakia	1103,39	1735,48	1312,54				6,47		4,54			
TOTAL	4038,47	3060,76	3530,73	188,03	356,94	414,43	15367,6	14973,32	20188,68	64,95	176,33	73,54

Table 1 (continued)

Codes in this table: IR: Iran; IN: India; MU: Mauritius; US: USA; VN: Vietnam

Export by:	IR	IR	IN	IN	IN	MU	US	US	US	VN	VN	VN
Import by :	2018	2020	2018	2019	2020	2019	2018	2019	2020	2018	2019	2020
Austria			0				37,48	2,56	0,92			
Belgium					17,46		0,04	2,07	0,19			
Bulgaria					0,56			0,01	400			
Cyprus							3,75	0,92	0,93			
Czechia			8,3	16,65	41,25		0,16	9,28	0,06	31,94	41,33	19,95
Germany		0,52	766,87	674,94	54,49		986,44	340,75	573,71		1,77	
Denmark		6,25			0,05		446,77	578,67	1766,4	11		50,5
Estonia							0,11	0,07	0,04			
Spain		3,4	36,48	74,01	35,23		116,94	236,82	123,33	2,29	0,24	0,65
Finland							0,22	0,39	0,28			
France			168,7	330,35	257,64		5061,7	6392,88	5558,66	23,19	3,67	4,41
UK			786,21	802,93	60,06		2239,44	1237,78	129,82			
Greece			3,61	0,12	0,22		16,69	17,78	11,99	0,4	0,13	0,09
Croatia				0			0,08	0,05	0,12			
Hungary			2,72	4,99	29,05		0,36	48,01	202,18			
Ireland			0	0,01	3,55		0,33	0,38	0,34			0,01
Italy	39		596,5	698,13	326,25		2373,8	2168,92	3497,53	0,08	13,94	1,49
Lithuania									0,05			
Luxembourg					0,18		0,02		0,01			
Latvia					0		0,03		0,1			
Malta							0,34	0,24	0,45			
Netherlands	30		1779,95	1081,1	740,51	0,07	17660,1	18720,64	19709,44	55,31	57,08	64,64
Poland			4,13	4,35	3,16		703,97	630,03	294,25			
Portugal				0,36	2,32		0,08	0,36	0,32		0	0,11
Romania			0,56	0,38	3,64		14,18	107,63	96,4			
Sweden				0,04	0,01		5,73	3,8	9,29	0,26		
Slovenia								0,02	0,03			
Slovakia								0,01				
TOTAL		10,17	4154,03	3688,36	1575,63	0,07	29631,28	30497,51	32375,92	124,47	118,16	141,85

Table 2. 07020000 Tomatoes, fresh or chilled

Codes in this table: CZ: Czech Rep.; IR: Iran; MX: Mexico; IN: India; US: USA.

Export by:	CZ	CZ	CZ	IR	MX	IN	US	US	US
Import by :	2018	2019	2020	2020	2020	2019	2018	2019	2020
Austria	1,28	55,56	96,45						
Bulgaria	537,78	356,28	2063,8	11,13					
Germany	936,9	120,44	75,75						
Denmark						0,01			
Spain	7,06	229,1	359,98						
France	31,44								
Croatia	138	222,72	480						
Hungary	6,8	501,32	2252,7						
Ireland							0,11		0,09
Italy		70,2	125,66						
Lithuania		65,26	89,2						
Netherlands	1114,01	863,24	368,86		0,8				
Poland	497,26	2869,81	162,12						
Romania	4295,1	2246,26	1049,33						
Sweden		72						0,04	0,04
Slovakia	60262,01	51368,02	38016,61						
TOTAL	67827,64	59040,21	45140,46	11,13	0,8	0,01	0,11	0,04	0,13

Table 3. 07096010 Fresh or chilled sweet peppers

Codes in this table: CZ: Czech Rep.; BR: Brazil; CN: China; IR: Iran; MX: Mexico; IN: India; MU: Mauritius; US: USA; VN: Vietnam.

Export by:	CZ	CZ	CZ	BR	CN	IR	MX	MX	MX	IN	IN	IN	MU	US	VN
Import by :	2018	2019	2020	2018	2019	2019	2018	2019	2020	2018	2019	2020	2019	2020	2019
Austria	3564,25	4207,88	3915,35			125,95									
Bulgaria	172,56	108,08	224,46												
Germany	193,2	2466,41	7,63												
Spain	98,95	339,59	201,73												
France			8,75	2,08				96,79	75,11	497,75	1619,26	9,5	4,8	0,09	
UK							749,1								
Croatia	668,7	435,6													
Hungary	2152,9	5611,02	6548,32												
Ireland															0,05
Italy		291,42	131,22				9,5	21,64		326,65	1368,39	1682,78			
Netherlands	517,02	454,37	864,09								2,13	0,5			
Poland	676,51	461,04	380,02												
Romania	1556,28	333,58	364,96												
Slovenia					100										
Slovakia	44479,43	45909,88	51780,65												
TOTAL	54079,8	60618,87	64427,18	2,08	100	125,95	758,6	118,43	75,11	824,4	2989,78	1692,78	4,8	0,09	0,05

Table 4. 07096099 Fresh or chilled fruits of genus *Capsicum* or *Pimenta* (excl. for industrial manufacture of capsin or capsin oleoresin dyes, for industrial manufacture of essential oils or resinoids, and sweet peppers)

Codes in this table: CZ: Czech Rep.; BR: Brazil; CN: China; IR: Iran; MX: Mexico; IN: India; US: USA; VN: Vietnam.

	CZ	CZ	CZ	BR	BR	CN	CN	CN	IR	IR	MX	MX	MX	IN	IN	IN	US	US	VN
	2018	2019	2020	2018	2019	2018	2019	2020	2018	2020	2018	2019	2020	2018	2019	2020	2018	2020	2018
AT	757,75	1313,88	1183,6																
BE																5	0,19		
BG									8,12										
CZ										0,3				1,5					1500,77
DE	10,13	70,14	83,53			0	0,05	0,34	5,3	53,74	95,17	228,7	1159,25	2024,05	2388,09	2,68	11	7,5	
DK											34,66								
ES										21,6				0					
FR	3,31			9,23						66,12		28,76	867,05	751,57	1139,7				
UK	25	22		17,86						1791,06	1721,34	324,39	11865,76	13399,07	2172,03				
HU								159											
IR											0,07				0,35		0,08		
IT													560,74	968,83	559,42				
NL	20,22	13,84	13,22		0,01						179,95	107,41	285,07	158,11	142,59		0,09		
PL	0,97	280,5																	
PT				0,69	0,1										6,51				
SE						13,76		2,84						58,11	105,22	143,03		0,02	
SK	3644,45	1257,35	1404,63																
	4461,83	2957,71	2684,98	27,78	0,11	13,76	0,05	162,18	5,3	8,12	1932,82	2031,19	689,26	14797,48	17413,36	6550,21	2,87	11,19	1508,27