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Pathogen profile

Tomato chlorosis virus, an emergent plant virus still expanding its geographical and host ranges

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SUMMARY

Tomato chlorosis virus (ToCV) causes an important disease that primarily affects tomato, although it has been found infecting other economically important vegetable crops and a wide range of wild plants. First described in Florida (USA) and associated with a 'yellow leaf disorder' in the mid-1990s, ToCV has been found in 35 countries and territories to date, constituting a paradigmatic example of an emergent plant pathogen. ToCV is transmitted semipersistently by whiteflies (Hemiptera: Aleyrodidae) belonging to the genera *Bemisia* and *Trialeurodes*. Whitefly transmission is highly efficient and cases of 100% infection are frequently observed in the field. To date, no resistant or tolerant tomato plants are commercially available and the control of the disease relies primarily on the control of the insect vector.

Taxonomy: *Tomato chlorosis virus* is one of the 14 accepted species in the genus *Crinivirus*, one of the four genera in the family *Closteroviridae* of plant viruses.

Virion and genome properties: The genome of ToCV is composed of two molecules of single-stranded positive-sense RNA, named RNA1 and RNA2, separately encapsidated in long, flexuous, rod-like virions. As has been shown for other closteroviruses, ToCV virions are believed to have a bipolar structure. RNA1 contains four open reading frames (ORFs) encoding proteins associated with virus replication and suppression of gene silencing, whereas RNA2 contains nine ORFs encoding proteins putatively involved in encapsidation, cell-to-cell movement, gene silencing suppression and whitefly transmission.

Host range: In addition to tomato, ToCV has been found to infect 84 dicot plant species belonging to 25 botanical families, including economically important crops.

Transmission: Like all species within the genus *Crinivirus*, ToCV is semipersistently transmitted by whiteflies, being one of only two criniviruses transmitted by members of the genera *Bemisia* and *Trialeurodes*.

Disease symptoms: Tomato 'yellow leaf disorder' syndrome includes interveinal yellowing and thickening of leaves. Symptoms first develop on lower leaves and then advance towards the upper part of the plant. Bronzing and necrosis of the older leaves are accompanied by a decline in vigour and reduction in fruit yield. In other hosts the most common symptoms include interveinal chlorosis and mild yellowing on older leaves.

Control: Control of the disease caused by ToCV is based on the use of healthy seedlings for transplanting, limiting accessibility of alternate host plants that can serve as virus reservoirs and the spraying of insecticides for vector control. Although several wild tomato species have been shown to contain genotypes resistant to ToCV, there are no commercially available resistant or tolerant tomato varieties to date.

Keywords: *Closteroviridae*, criniviruses, emergent diseases, tomato, tomato chlorosis virus, whiteflies.

DISCOVERY AND EMERGENCE OF TOCV

The family *Closteroviridae* of plant viruses includes the genera *Ampelovirus*, *Closterovirus*, *Crinivirus*, *Velarivirus*, and a few unassigned species. The genus *Crinivirus* includes the 14 whitefly-transmitted members of the family (ICTV, 2018). Most individual criniviruses cause significant diseases in important crops including common bean, beet, cucurbits, lettuce, potato and tomato, whereas some of them are asymptomatic and only cause disease when found in mixed infections with other viruses (Tzanetakis *et al.*, 2013).

Tomato chlorosis virus (ToCV), a crinivirus, was first identified in the mid-1990s from greenhouse-grown tomato plants showing a 'yellow leaf disorder' syndrome in north-central Florida, USA. This syndrome had been observed since 1989 but was initially attributed to physiological or nutritional disorders (Wisler *et al.*, 1998a, 1998b). Symptoms observed in ToCV-infected tomato plants include chlorotic areas that evolve into interveinal bright yellowing, beginning on lower leaves and gradually progressing

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to the upper part of the plant (Fig. 1). In the initial stage of the infection, chlorotic areas are frequently polygonal in shape, which is limited by main veins. In advanced stages, interveinal yellow areas can develop reddish-brown necrotic flecks. Lower leaves present as rolled, thickened and brittle, being crispy to the touch. Although no obvious symptoms are produced on fruits, significant yield reduction occurs due to a loss of photosynthetic area. Symptomatic plants are less vigorous, showing yield losses as a result of reduced fruit growth and delayed ripening. Symptoms of ToCV on tomato are indistinguishable from those induced by a related crinivirus, tomato infectious chlorosis virus (TICV) (Duffus *et al.*, 1996). In other hosts, the most common symptoms caused by ToCV include interveinal chlorosis or mild yellowing on older leaves.

Soon after tomato yellow leaf disorder was recognized as being caused by a new crinivirus in the USA, similar symptoms were detected in Spain (Navas-Castillo *et al.*, 2000). These symptoms were observed as early as 1997 and its association with ToCV infection was confirmed for the first time in Europe. Since then, the virus has been detected infecting tomato in

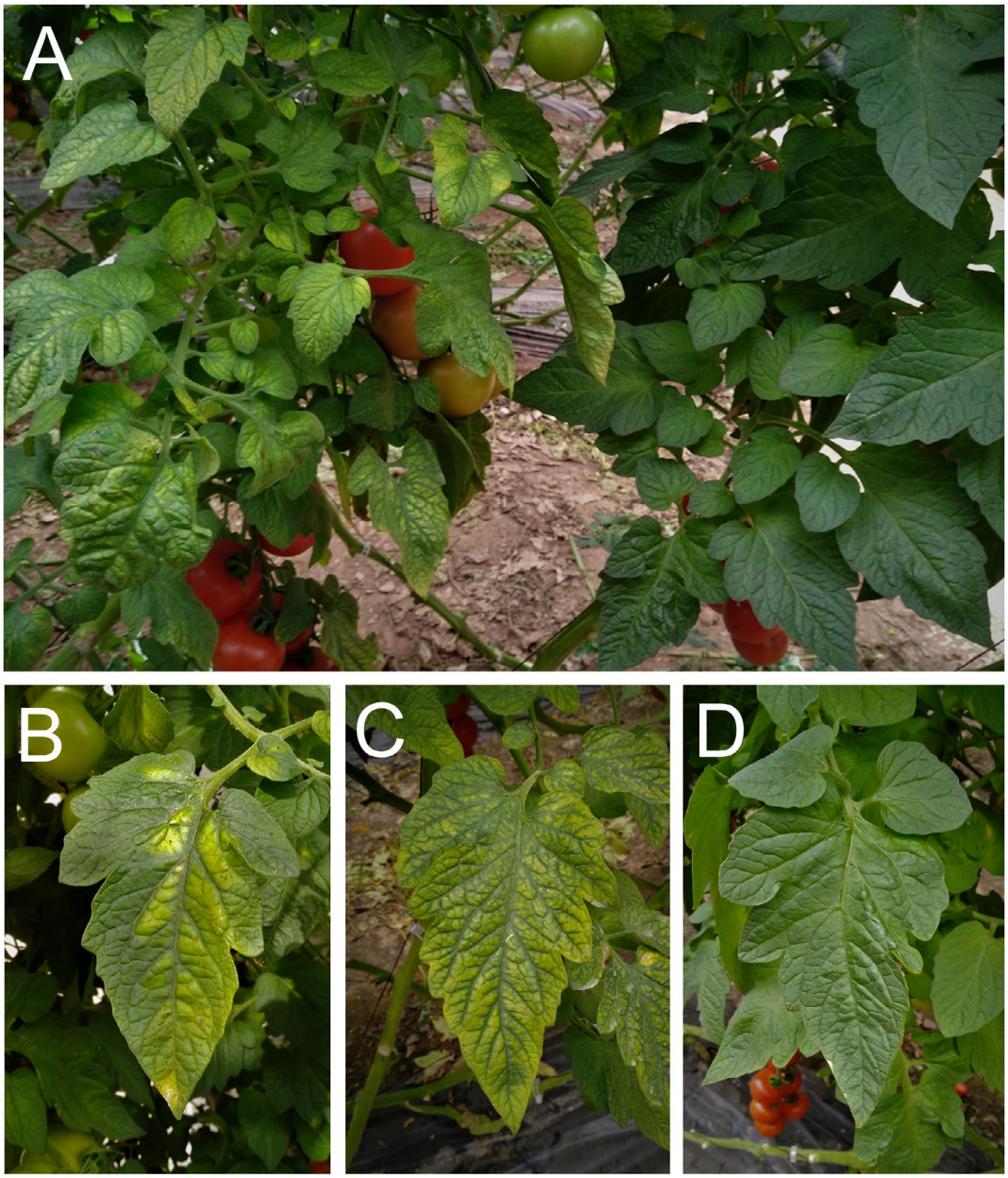

Fig. 1 Symptoms of tomato chlorosis virus infection on naturally infected tomato plants. (A) Commercial plant showing generalized yellowing (left) close to a healthy plant (right). Individual leaflets showing polygonal chlorotic areas (B) or interveinal bright yellowing (C) are shown alongside an asymptomatic leaflet (D).

many areas around the world. Altogether, ToCV has been found in 35 countries and territories in the Americas, Europe, Asia and Africa (Table 1, Fig. 2). During the 22 years since the presence of ToCV in the USA was confirmed, a linear progression of the disease has been observed throughout the world. This expansion is still evident; overall, a mean of 1.5 new countries or territories have been invaded per year (Fig. 2). Confirmation of viral presence in new areas could be dependent on a greater research effort on ToCV detection and identification. However, it is evident that recognition of new symptoms in a given area by farmers, who used to be the main actors in initial warnings given to scientists, has been occurring in new countries in recent years. In this sense, ToCV constitutes a paradigmatic example of an emergent plant pathogen that will surely invade new areas in the near future. It is worth noting the recent detection of ToCV in northern Europe, specifically in the Netherlands and the UK (EPPO, 2018a, 2018b). The climatic conditions in these countries were not thought to be conducive to the transmission of the virus, and its spread here was not foreseen. Cultivation of tomato under greenhouses, together with the fact that ToCV can be transmitted by *Bemisia tabaci* and *Trialeurodes vaporariorum*, the latter less dependent on warm climates than *B. tabaci*, means that the infections could spread to new areas. Climate change could aggravate this situation as the geographical range, phenology, density and activity of the vectors could be altered (Canto *et al.*, 2009). Another worrisome situation is related to the recent report of ToCV in Nigeria (Mohammed *et al.*, 2018), where the disease has been observed simultaneously in seven states of the country. Expansion of the virus to other tropical countries in sub-Saharan Africa will alter the scenario in this huge area, whose agriculture is already seriously threatened by other insect-borne viruses.

Table 1 Dates and references for first reports of tomato chlorosis virus in the countries and territories showed in Fig. 2

Year*	Country/territory	Reference
1996	United States	Wisler *et al.* (1998b)
1997	Spain	Navas-Castillo *et al.* (2000)
1998	Portugal	Louro *et al.* (2000)
1998	Taiwan	Tsai *et al.* (2004)
2000	Italy	Accotto *et al.* (2001)
2000	Morocco	Hanafi (2002)
2000	South Africa	Jones (2001)
2001	Canary Islands (ES)	Font *et al.* (2003)
2001	Greece	Dovas *et al.* (2002)
2001	Puerto Rico (US)	Wintermantel *et al.* (2001)
2002	France	Dalmon *et al.* (2005)
2003	Israel	Segev *et al.* (2004)
2004	Cyprus	Papayiannis *et al.* (2006)
2004	Réunion (FR)	Delatte *et al.* (2006)
2005	Mayotte (FR)	Massé *et al.* (2008)
2005	Mexico	Alvarez-Ruiz *et al.* (2007)
2006	Brazil	Barbosa *et al.* (2008)
2006	Cuba	Martínez-Zubiaur *et al.* (2008)
2006	Lebanon	Abou-Jawdah *et al.* (2006)
2007	Costa Rica	Castro *et al.* (2009)
2007	Mauritius	Lett *et al.* (2009)
2008	Japan	Hirota *et al.* (2010)
2008	Turkey	Çevik and Erkiş (2008)
2008	Indonesia	Suastika *et al.* (2011)
2010	Hungary	Bese *et al.* (2011)
2011	Sudan	Fiallo-Olivé *et al.* (2011)
2012	China	Zhao *et al.* (2013)
2012	Uruguay	Arruabarrena *et al.* (2014)
2013	Korea	Lee *et al.* (2018)
2014	Tunisia	Mnari-Hattab *et al.* (2014)
2014	Saudi Arabia	Al-Saleh *et al.* (2014)
2014	Jordan	Salem *et al.* (2015)
2017	Nigeria	Mohammed *et al.* (2018)
2017	Netherlands	EPPO (2018a)
2018	United Kingdom	EPPO (2018b)

ES, Spain; US, United States; FR, France.

*Whenever it could be determined from the literature, dates correspond to collection of samples that were confirmed to be infected.

In addition to tomato, ToCV has been found infecting a high number of diverse plant species. Overall, 85 dicot plant species belonging to 25 botanical families have been described as natural hosts for the virus (Table 2). These hosts include economically important vegetable (cowpea, eggplant, lettuce, potato, pumpkin, sweet pepper and tomato) and ornamental crops, as well as weeds and other wild plants. For their global importance, sweet pepper and potato crops can be highlighted in addition to tomato. ToCV infections on sweet pepper have been reported from Spain (Lozano *et al.*, 2004), Brazil (Barbosa *et al.*, 2010), Costa Rica (Vargas *et al.*, 2011), Tunisia (Gharsallah *et al.*, 2015) and Saudi Arabia (Shakeel *et al.*, 2017). The effect of the disease on sweet pepper has been revealed by controlled experiments using *B. tabaci* as a vector (Fortes *et al.*, 2012). These experiments showed that varieties of the three basic types of pepper grown in the Mediterranean basin (Italian, California and Lamuyo) are efficiently infected by the virus. Also, in addition to confirming the development of the symptoms observed in the field (stunting growth of the plant, curling, interveinal yellowing and abnormal elongation in leaves), an important decrease of marketable fruit yield was observed due to reduction in fruit number and size (Fig. 3). Potato plants have been also shown to be naturally infected by

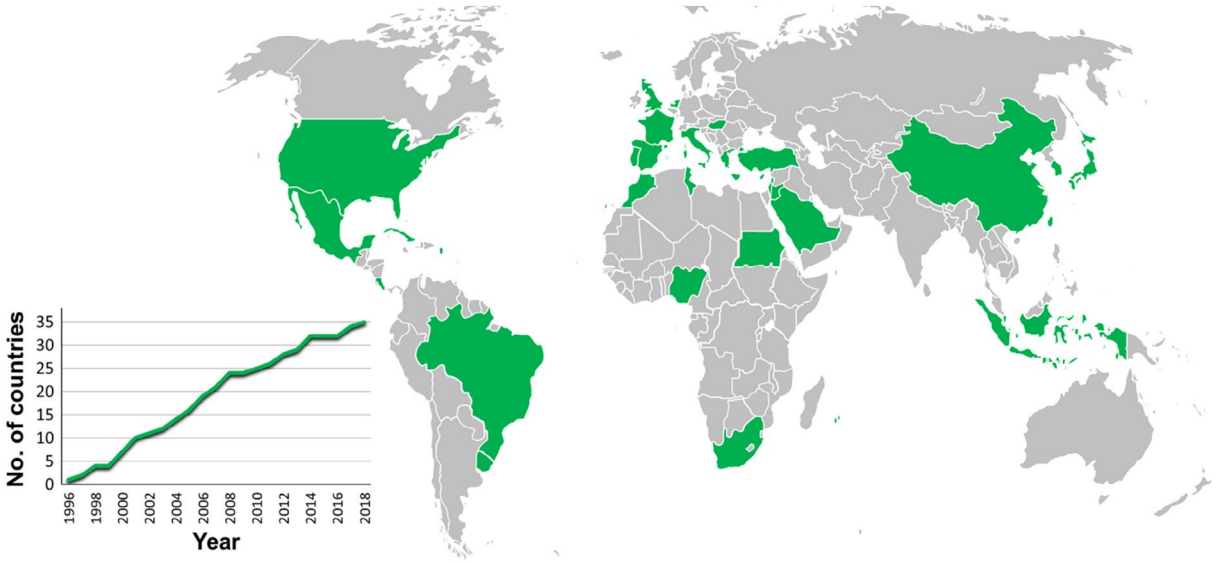

Fig. 2 Geographical distribution of tomato chlorosis virus (ToCV). A complete list of countries and territories where the virus has been reported (in green) and references for first reports are shown in Table 1. The inset graph shows the evolution through time of the number of countries and territories where ToCV has been found to date.

ToCV in Spain (Fortes and Navas-Castillo, 2008, 2012), Brazil (Freitas *et al.*, 2012) and Tunisia (Gharsallah *et al.*, 2015). Transmission experiments have shown the presence of ToCV in potato tubers from infected plants, which subsequently produced infected plants themselves, and that this species served as virus source for tomato infection via *B. tabaci* transmission (Fortes and Navas-Castillo, 2012). Although the above experiments did not show any symptoms in the ToCV-infected potato cv. Safrane plants, plants of cv. Ágata reported as being infected by ToCV in Brazil showed leaf roll and interveinal chlorosis symptoms on older leaves (Freitas *et al.*, 2012).

GENOME ORGANIZATION AND DIVERSITY

To date, 17 complete ToCV genome sequences have been published, one each from the USA (Wintermantel *et al.*, 2005), Spain (Lozano *et al.*, 2006b, 2007), Greece (Kataya *et al.*, 2008), Brazil (Albuquerque *et al.*, 2013) and Taiwan (Kang *et al.*, 2018), two from China (Zhao *et al.*, 2014a, 2014b) and ten from Korea (Lee *et al.*, 2018).

The ToCV genome has the typical organization of bipartite criniviruses, with two molecules of linear, positive-sense, single-stranded RNA, namely RNA1 (8593–8596 nt) and RNA2 (8242–8247 nt) (Lozano *et al.*, 2006b, 2007; Wintermantel *et al.*, 2005) (Fig. 4). RNA1 contains four open reading frames (ORF 1a/1b to ORF3) potentially encoding proteins associated with virus replication and the suppression of gene silencing. RNA2 contains nine ORFs (ORF4 to ORF12) possibly encoding proteins putatively involved in virus encapsidation, cell-to-cell movement, membrane association, whitefly transmission and the suppression of gene silencing. Both RNAs, encapsidated in separated flexuous rod particles of approximately 800–850 nm in length (Liu *et al.*, 2000), are needed for infectivity (Orílio *et al.*, 2014). Although there is no direct evidence, it is supposed that ToCV virions have the rattlesnake structure described for the crinivirus lettuce infectious yellows virus (LIYV), composed of a long body and a short tail (Tian *et al.*, 1999). This feature is common to all members of the family *Closteroviridae* (Agranovsky *et al.*, 1995).

ORF 1a encodes a 221 kDa multifunctional protein typical of members of the family *Closteroviridae* (Karasev, 2000), containing protease, methyltransferase and helicase domains (Wintermantel *et al.*, 2005). ORF 1b expresses a 59 kDa RNA-dependent RNA polymerase (RdRp) likely through a +1 ribosomal frameshift at the end of ORF 1a, as happens in other members of the family *Closteroviridae*, although no specific structures that favour the slippage have been identified (Wintermantel *et al.*, 2005). ORF 2 encodes p22, a protein with little similarity to other proteins in searched databases, although an ORF of similar size and location is present in the genome of other criniviruses. The p22 protein has been shown to be an efficient and strong RNA silencing suppressor by the classical *Agrobacterium* co-infiltration assay on *Nicotiana benthamiana* plants, in which it has an extremely long-lasting local activity (Cañizares *et al.*, 2008). It has been suggested that the suppression mechanism of p22 is mediated by its preferential binding to long dsRNAs, preventing them from being cleaved (Landeo-Ríos *et al.*, 2016). ORF 3 encodes a putative protein of 6 kDa (p6) with a transmembrane domain similar to other 3′-end proteins of criniviruses.

ORF 4 encodes a putative 4 kDa protein (p4) with a hydrophobic domain, suggesting it functions as a transmembrane protein. Similarly sized hydrophobic proteins are putatively encoded

Table 2 Natural hosts reported for tomato chlorosis virus.

Family	Plant species*	Reference
Aizoaceae	*Heliotropium lasiocarpum*	Shakeel *et al.* (2017)
Amaranthaceae	*Alternanthera philoxeroides*	Tang *et al.* (2017)
	Amaranthus retroflexus	Orfanidou *et al.* (2014)
	Amaranthus viridis	Shakeel *et al.* (2017)
Apocynaceae	*Calotropis procera*	Shakeel *et al.* (2017)
Araliaceae	*Aralia nudicaulis*	Shakeel *et al.* (2017)
Asteraceae	*Cirsium arvense*	Orfanidou *et al.* (2014)
	Conyza canadensis	Kil *et al.* (2015b)
	Conyza sp.	Orfanidou *et al.* (2014)
	Erigeron annuus	Kil *et al.* (2015a)
	Lactuca saligna	Shakeel *et al.* (2017)
	***Lactuca sativa* (lettuce)**	Orfanidou *et al.* (2014)
	Lactuca serriola	Shakeel *et al.* (2017)
	Sonchus asper	Kil *et al.* (2015b)
	Sonchus olereaceus	Shakeel *et al.* (2017)
	Youngia japonica	Kil *et al.* (2015b)
Boraginaceae	*Trigonotis peduncularis*	Kil *et al.* (2015b)
Brassicaceae	***Brassica* sp.**[†]	Solórzano-Morales *et al.* (2011)
	Cardamine flexuosa	Kil *et al.* (2015b)
	***Eruca vesicaria* (garden rocket)**	Boiteux *et al.* (2016)
	***Raphanus raphanistrum* (radish)**	Boiteux *et al.* (2016)
Caryophyllaceae	*Cerastium glomeratum*	Kil *et al.* (2015b)
	Stellaria media	Kil *et al.* (2015b)
Chenopodiaceae	*Chenopodium album*	Orfanidou *et al.* (2014)
	Chenopodium murale	Shakeel *et al.* (2017)
	Chenopodium opulifolium	Shakeel *et al.* (2017)
Compositae	***Zinnia elegans* (zinnia)**	Tsai *et al.* (2004)
Convolvulaceae	*Convolvulus arvensis*	Orfanidou *et al.* (2014)
	Ipomoea cholulensis	Kil *et al.* (2015b)
	Ipomoea hederaceae	Kil *et al.* (2015b)
Cucurbitaceae	***Cucurbita moschata* (pumpkin)**	Solórzano-Morales *et al.* (2011)
Fabaceae	*Vicia angustifolia*	Kil *et al.* (2015b)
	Vicia tetrasperma	Kil *et al.* (2015b)
	***Vigna unguiculata* (cowpea)**	Wang *et al.* (2018a)
Fumariaceae	*Fumaria officinalis*	Orfanidou *et al.* (2014)
Malvaceae	***Abelmoschus esculentus* (okra)**	Shakeel *et al.* (2017)
	Abutilon theophrasti	Orfanidou *et al.* (2014)
	Malva parviflora	Shakeel *et al.* (2017)
	Malva sylvestris	Orfanidou *et al.* (2014)
Mazaceae	*Mazus pumilus*	Kil *et al.* (2015b)
Oxalidaceae	*Oxalis pes-caprae*	Orfanidou *et al.* (2014)
Phytolacaceae	*Phytolacca americana*	Kil *et al.* (2015b)
	Phytolacca icosandra	Solórzano-Morales *et al.* (2011)

(Continues)

Table 2 (Continued)

Family	Plant species*	Reference
Plantaginaceae	*Plantago major*	Solórzano-Morales *et al.* (2011)
	Veronica hederifolia	Orfanidou *et al.* (2014)
Portulacaceae	***Portulaca oleracea* (purslane)**‡	Orfanidou *et al.* (2014)
Primulaceae	*Anagalis foemina*	Orfanidou *et al.* (2014)
Rubiaceae	*Galium aparine*	Orfanidou *et al.* (2014)
Rutaceae	*Ruta chalepensis*	Solórzano-Morales *et al.* (2011)
Solanaceae	***Capsicum annuum* (sweet pepper)**	Lozano *et al.* (2004)
	Datura stramonium	Alvarez-Ruiz *et al.* (2007)
	Nicandra physaloides	Souza *et al.* (2019)
	***Nicotiana tabacum* (tobacco)**	Fiallo-Olivé *et al.* (2014)
	Physalis angulata	Fonseca *et al.* (2013)
	Physalis ixocarpa	Trenado *et al.* (2007)
	Physalis peruviana	Trenado *et al.* (2007)
	***Solanum aethiopicum* (scarlet eggplant)**	Fonseca *et al.* (2016)
	Solanum americanum	Arruabarrena *et al.* (2015)
	Solanum arcanum	García-Cano *et al.* (2010)
	Solanum chilense	García-Cano *et al.* (2010)
	Solanum chmielewskii	García-Cano *et al.* (2010)
	Solanum corneliomulleri	García-Cano *et al.* (2010)
	Solanum elaeagnifolium	Gharsallah *et al.* (2015)
	Solanum galapagense	García-Cano *et al.* (2010)
	Solanum habrochaites	García-Cano *et al.* (2010)
	Solanum huaylasense	García-Cano *et al.* (2010)
	Solanum jamaicense	Boiteux *et al.* (2018)
	***Solanum lycopersicum* (tomato)**	Wisler *et al.* (1998b)
	Solanum mammosum	Boiteux *et al.* (2018)
	***Solanum melongena* (eggplant)**	Zhou *et al.* (2015)
	Solanum neorickii	García-Cano *et al.* (2010)
	Solanum nigrescens	Alvarez-Ruiz *et al.* (2007)
	Solanum nigrum	Font *et al.* (2004)
	Solanum paniculatum	Boiteux *et al.* (2018)
	Solanum pennellii	García-Cano *et al.* (2010)
	Solanum peruvianum	García-Cano *et al.* (2010)
	Solanum pimpinellifolium	García-Cano *et al.* (2010)
	Solanum scuticum	Boiteux *et al.* (2018)
	Solanum sessiliflorum	Boiteux *et al.* (2018)
	Solanum sisymbriifolium	Arruabarrena *et al.* (2015)
	Solanum stramoniifolium	Boiteux *et al.* (2018)
	Solanum subinerme	Boiteux *et al.* (2018)
	***Solanum tuberosum* (potato)**	Fortes and Navas-Castillo (2008)
	Solanum velleum	Boiteux *et al.* (2018)
Zygophyllaceae	*Tribulus terrestris*	Shakeel *et al.* (2017)

*For cultivated hosts (highlighted in bold), the common name is given in parentheses after the scientific name.†The infected *Brassica* plants found in Costa Rica, unidentified to the species level, were reported as weeds.‡Although purslane is grown in many areas, the infected samples from Greece were reported as weeds.

in this location in most other criniviruses, although comparison of the amino acid sequences does not reveal obvious similarity for these proteins, with the possible exception of sweet potato chlorotic stunt virus (Kreuze *et al.*, 2002). ORF 5 encodes a heat shock protein 70 homologue (HSP70h) that likely associates with virion tails and is involved in their assembly, along with cell-to-cell movement based on similarity with the closteroviruses beet yellows virus (BYV) and citrus tristeza virus (Alzhanova *et al.*, 2001; Napuli *et al.*, 2000; Peremyslov *et al.*, 1999; Satyanarayana *et al.*, 2000). ORF 6 encodes a putative 8 kDa protein (p8) with similarity to 6 kDa proteins encoded by beet pseudoyellows virus and strawberry pallidosis-associated virus (Tzanetakis and Martin, 2004; Tzanetakis *et al.*, 2005). ORF 7 encodes a 59 kDa protein (p59) that also associates with virion tails and is likely involved in cell-to-cell movement (Alzhanova *et al.*, 2001; Satyanarayana *et al.*, 2000). ORF 8 encodes a putative 9 kDa protein (p9) for which no function or similarity has been identified. ORFs 9 and 10 encode 29 kDa and 76 kDa major and minor coat proteins (CP and CPm), respectively. As is typical of all members of the family *Closteroviridae*, the CP encapsidates most of the virion particle, while CPm is associated with the virion tail (Agranovsky *et al.*, 1995). Studies on LIYV have suggested that CPm may also be involved in virus transmission by the whitefly vectors (Chen *et al.*, 2011; Stewart *et al.*, 2010). CP and CPm may also be involved in cell-to-cell movement based on studies conducted with BYV (Alzhanova *et al.*, 2000, 2001). ToCV CP and CPm have also been shown to have silencing suppression activity (Cañizares *et al.*, 2008). ORF 11 encodes a 27 kDa protein (p27) which seems unique to criniviruses. ORF 12 encodes a putative 7 kDa protein (p7) unique to ToCV among criniviruses characterized to date. This putative protein contains a transmembrane domain, which suggests it may be a functional protein, although no similarity has been found to any other protein in the searched databases.

ToCV populations have a heterogeneous and complex genetic structure similar to those described for animal and plant RNA viral quasispecies. This was shown by analysing the intraisolate genetic structure of 755 molecular clones distributed in four genomic regions within the RNA-dependent RNA polymerase (RNA1) and HSP70h, CP and CPm (RNA2) ORFs of ToCV isolates from Spain (Lozano *et al.*, 2009). Also, it has been shown that the structure of the ToCV populations clearly differ depending on the RNA segment considered, being more complex for RNA1 (encoding replication-associated proteins) than for RNA2 (encoding encapsidation, systemic-movement and insect transmission-relevant proteins) (Lozano *et al.*, 2009; our unpublished results). This observation supports the idea that, in multicomponent RNA viruses, function can generate profound differences in the genetic structures of the different genomic segments.

A few studies have shown a low genetic diversity of ToCV when analysing partial sequences (HSP70h, CP and CPm genes) within a country (Barbosa *et al.*, 2013; Orfanidou *et al.*, 2014). However, when all available full-length genome sequences have been compared, three major clusters can be recognized (Lee *et al.*, 2018).

Availability of infectious clones

Orílio *et al.* (2014) constructed full-length cDNA infectious clones for both RNA1 and RNA2 of a Spanish ToCV isolate, AT80/99. *In vitro* transcripts of the RNA1 clone, under the control of SP6 RNA promoter, were able to replicate in *N. benthamiana* mesophyll protoplasts. On the other hand, *in vivo* transcripts of the RNA1 and RNA2 clones, under the control of 35S CaMV promoter, resulted in infection on *N. benthamiana* plants after agroinoculation. As has been reported for other criniviruses, infection could not be achieved directly on the natural host of the virus, tomato. However, grafting of agroinfected *N. benthamiana* stem scions onto tomato plants allowed systemic infection of tomato plants, which then showed the characteristic yellowing symptoms caused by ToCV. The viruses generated in tomato were shown to be whitefly transmitted, both by *B. tabaci* Mediterranean (MED, formerly Q biotype) and Middle East-Asia Minor 1 (MEAM1, formerly B biotype), thus proving the functional integrity of the virus progeny generated from the infectious clones. Full-length cDNA clones under a 35S CaMV promoter have been also obtained for a Chinese ToCV isolate, whose biological activity was demonstrated through agroinoculation of *N. benthamiana* plants (Zhao *et al.*, 2016). A recent paper has reported that the latter clones resulted infectious in tomato plants, although details on the inoculation method used or the efficiency of infection were not given (Shi *et al.*, 2018).

EPIDEMIOLOGY

Criniviruses emerged as a major problem for world agriculture at the end of the twentieth century with the establishment of some of their whitefly vectors in temperate climates. ToCV is one of two criniviruses that are transmitted by whiteflies of the genera *Bemisia* and *Trialeurodes*. These include several species of the *B. tabaci* complex [MED, MEAM1 and New World (NW, formerly A biotype)], *T. vaporariorum* and *T. abutilonea* (Navas-Castillo *et al.*, 2000; Wisler *et al.*, 1998b). The high number of natural plant hosts (see Table 2) and being readily transmitted by several whitefly species have contributed to successful emergence of ToCV worldwide (see Table 1 and Fig. 2). In Spain, for example, ToCV prevalence in tomato crops is reported to be very high, frequently at levels of 50–100% (Fortes *et al.*, 2012; Lozano *et al.*, 2006a; Velasco *et al.*, 2008). Field investigations conducted in Brazil on tomato have shown that the main dispersal mechanism

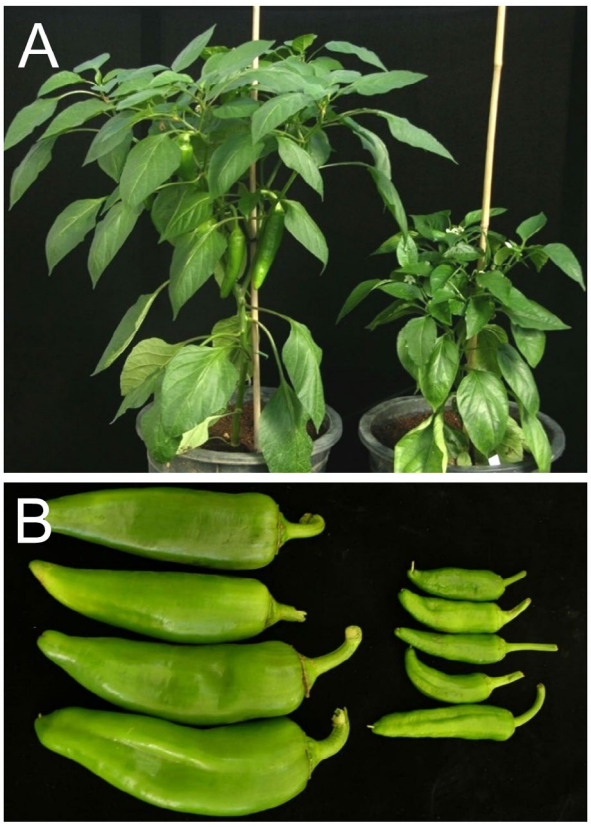

Fig. 3 Effect of tomato chlorosis virus infection on pepper (cv. Pescara) plants inoculated using viruliferous *Bemisia tabaci* and maintained under controlled conditions. (A) Plant growth reduction in an infected plant (right) in comparison to a mock-inoculated plant (left). (B) Reduction in size of fruits from infected plants (right) in comparison to those from mock-inoculated plants (left). Reproduced from Fortes *et al.* (2012).

of the disease caused by ToCV is primary spread, with epidemics being caused by successive influxes of viruliferous whiteflies (Macedo *et al.*, 2019).

The presence of wild and cultivated (e.g. sweet pepper and potato) alternative hosts for ToCV (Table 2) adds a new dimension to the complexity of its epidemiology. Although there are no in-depth studies regarding the epidemiological consequences of wild host presence or the coincidence of tomato with sweet pepper, potato or other susceptible crops in the field, some insights can be gained from laboratory experiments. Thus, both choice and non-choice transmission experiments using *B. tabaci* MEAM1 as a vector and tomato or potato plants as source of inoculum have shown that ToCV transmission rates follow the order tomato > potato > sweet pepper (Mituti *et al.*, 2018). These studies also showed that tomato is a better source of inoculum than potato.

Whitefly transmission

ToCV is transmitted semipersistently and, based on similarity with LIYV (Chen *et al.*, 2011), is believed to be foregut-borne. Although it is commonly accepted that ToCV is a phloem-limited virus, only recently has it been shown using the electrical penetration graph technique that transmission occurs primarily after salivation activity in the phloem sieve elements (waveform E_1) (Maluta *et al.*, 2017). Unexpectedly, this investigation suggests that a very low rate of infection (below 2%) could also occur before salivation during stylet puncture in mesophyll, companion or parenchyma cells, without ruling out brief stylet punctures in phloem sieve elements. Similar results have been observed with lettuce chlorosis virus, another crinivirus, with a low rate of

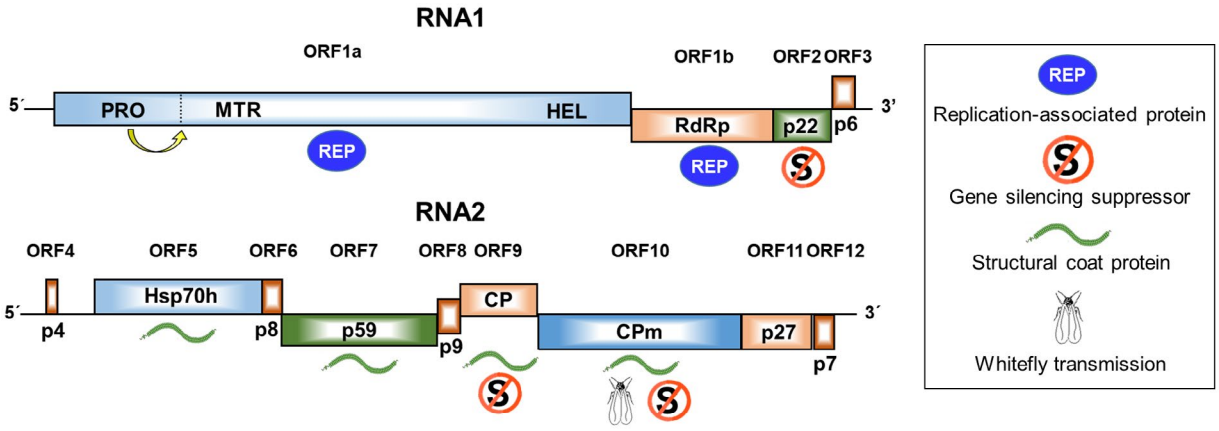

Fig. 4 Schematic representation of the genomic structure of tomato chlorosis virus RNA1 and RNA2. Boxes represent open reading frames (ORF) with the putative protein products indicated inside. The inset shows the symbols used to represent the (putative) functions of proteins: replication-associated proteins, gene silencing suppresors, structural coat proteins and whitefly transmission. CP, coat protein; CPm, minor coat protein; HSP70h, heat shock protein 70 homologue; HEL, helicase; MTR, methyl transferase; PRO, proteinase, RdRp, RNA-dependent RNA polymerase.

transmission before whiteflies performed waveform E_1 (Johnson *et al.*, 2002).

The transmission efficiency differs among the whitefly species, following the order *B. tabaci* MED > *B. taba*ci MEAM1 ≈ *T. abutilonea* > *B. tabaci* NW > *T. vaporariorum* (Shi *et al.*, 2018; Wintermantel and Wisler, 2006). The difference in transmission between MED and MEAM1, the most invasive species in the *B. tabaci* complex, has been associated to differences in virus acquisition and accumulation rate (Shi *et al.*, 2018).

Virus–plant–whitefly interactions

Recently, a number of papers have been published describing the interactions between ToCV, tomato plants and whitefly vectors. The parameters most commonly studied include preference and performance of the insects on infected and healthy plants. The results of these studies are apparently contradictory, even in cases where they were carried out with the same whitefly species, suggesting differences in the experimental conditions. Thus, two studies with *B. tabaci* MEAM1 reached different conclusions regarding the preference of adults for virus-infected tomato leaves in comparison to healthy leaves; the preference for virus-infected plants was shown only for non-viruliferous adults by Shi *et al.* (2018) while Fereres *et al.* (2016) found preference for both viruliferous and non-viruliferous adults. Also, studies on performance of whiteflies on ToCV-infected plants, as determined by fecundity and other reproductive parameters, resulted in contrasting conclusions. Thus, according to Shi *et al.* (2018), *B. tabaci* MED adults performed better on infected plants while according to Li *et al.* (2018), they performed worst. On the other hand, *B. tabaci* MEAM1 adults performed similarly on infected and healthy plants according to Shi *et al.* (2018) and Maluta *et al.* (2019), while they performed worst on infected plants according to Watanabe *et al.* (2018). Although the effects of plant pathogens on the behaviour and biological performance of their vectors have been shown for different host–pathogen–vector combinations, from the available studies carried out with ToCV it is clear that additional research is needed to correctly understand the interactions between the virus, the plant host and whiteflies, and their further epidemiological implications.

Mixed infections and synergism

Mixed virus infections are frequently found in wild and cultivated plants. In some cases, the interaction between two or more viruses results in a synergistic interaction. Synergism has a facilitative effect on one or all of the viral partners and can be manifested by an increase in virus replication, symptomatology, cellular tropism, within host movement and transmission rate (reviewed by Syller, 2012).

In the case of ToCV in tomato, mixed infections with other viruses have been described to be frequent in several countries. The viruses involved in mixed infections with ToCV include tomato yellow leaf curl virus (a monopartite Old World begomovirus transmitted by *B. tabaci*) (Martínez-Zubiaur *et al.*, 2008), tomato severe rugose virus (a bipartite New World begomovirus transmitted by *B. tabaci*) (Macedo *et al.*, 2014), pepino mosaic virus (a potexvirus transmitted by mechanical contact) (Davino *et al.*, 2008) and tomato torrado virus (a torradovirus transmitted by *B. tabaci* and *T. vaporariorum*) (Gómez *et al.*, 2010). The above papers describing mixed infections with begomoviruses reported that the symptoms observed in plants infected with both are more severe than those induced by the begomovirus alone. Although these observations are suggestive of a synergistic interaction with a facilitative effect of ToCV on the begomovirus, to our knowledge there is no study available in which this phenomenon has been examined in depth.

On the other hand, the interaction between ToCV and tomato spotted wilt virus (TSWV) (an orthotospovirus transmitted by thrips) has been studied under experimental conditions (García-Cano *et al.*, 2006). A synergistic reaction was observed in mixed infections in tomato plants susceptible to both viruses when doubly infected with ToCV and TSWV, resulting in the rapid death of plants. Also, a pronounced enhancement of ToCV accumulation, but not of TSWV, was observed in mixed infections. Synergism was also observed in tomato plants carrying the *Sw-5* resistance gene, which are resistant to TSWV. Pre-infection with ToCV resulted in susceptibility to TSWV, whereas co-inoculations did not, suggesting that a threshold level or a time lapse is needed for ToCV to interfere or down-regulate the defence response in the TSWV-resistant plants.

CONTROL STRATEGIES

As ToCV cannot be transmitted from plant to plant in the absence of whitefly vectors, suppression of vector populations can keep virus dispersion to a minimum. However, chemical control of the vector, widely used by tomato growers in many areas, has not proven to be effective for control of the disease caused by ToCV. This is especially true for open-field cultivation. A few simple crop management strategies have been evaluated for controlling the disease damage based on limiting the access of the whitefly vectors to the plants. Thus, Velasco *et al.* (2008) compared the prevalence of tomato yellowing caused by ToCV in south-eastern Spain (Murcia Region) where different greenhouse or nethouse covers are commonly used. These covers include five categories ranging from nethouses with 6 × 6 threads per centimetre (equivalent to open-air cultivation with the only purpose of avoiding wind damage) to polycarbonate plastic greenhouses with 10 × 20 threads per centimetre window screens. Only when the higher quality covers are used was a significant reduction of the disease observed, as determined by the area under the

disease progress curve. Similarly, experiments were carried out in east China (Shandong Province) by Wang *et al.* (2018b) to compare the influence of different structures and covers on ToCV prevalence. In this case four types of greenhouse were assayed, and it was found that adding nets to the greenhouse ventilation windows reduced the level of ToCV infection. These two reports show that reducing entry of whiteflies to the greenhouses by using simple containment structures results in an efficient protection of the crop from ToCV infection.

Diagnostics

Availability of specific and sensitive diagnostic methods is a key factor for designing control strategies against plant pathogens. In the case of ToCV, both serological and molecular diagnostic methodologies have been developed. For serological detection of ToCV, a polyclonal antiserum produced using viral capsid protein expressed in *Escherichia coli* was proposed for routine diagnosis in double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) but about 5% of false negatives were reported to occur (Jacquemond *et al.*, 2009). Polyclonal antibodies are now available to be used in DAS-ELISA from a number of commercial sources [e.g. the German Collection of Microorganisms and Cell Cultures (DSMZ) and the company Loewe].

Several reverse transcription-polymerase chain reaction (RT-PCR) protocols have been developed to allow rapid and specific detection of ToCV. These include a multiplex RT-PCR assay which allows the simultaneous detection of ToCV and TICV by amplifying part of the HSP70h gene of both criniviruses followed by a multiplex nested PCR amplification (Dovas *et al.*, 2002). A real-time TaqMan RT-PCR assay has been also developed for the multiplex detection of ToCV and TICV in plants and vector whiteflies, with a higher sensitivity than the conventional RT-PCR test (Papayiannis *et al.*, 2011).

Molecular hybridization of petiole cross-section tissue printing has been used for ToCV detection in plants by using a digoxigenin-labelled RNA probe obtained from the ToCV CP gene (Fortes *et al.*, 2012; Trenado *et al.*, 2007). This probe has proved to be useful for diagnosis of ToCV in epidemiological studies and for evaluation of genetic resistance in wild tomato germplasm (García-Cano *et al.*, 2010; Gómez *et al.*, 2010).

In recent years, several reverse transcription loop-mediated isothermal amplification (RT-LAMP) protocols have been also developed to detect ToCV in plants and whiteflies (Karwitha *et al.*, 2016; Kil *et al.*, 2015a; Zhao *et al.*, 2015).

Search for genetic resistance

To date, there is no commercial tomato cultivar reported to be resistant or tolerant to ToCV. However, experiments carried out in Spain, Brazil and Uruguay have identified several wild tomato species (*Solanum* section *Lycopersicon*) that contain genotypes reacting with mild symptoms and/or low viral titres to the natural or experimental inoculation with ToCV (García-Cano *et al.*, 2010; González-Arcos *et al.*, 2018; Mansilla-Córdova *et al.*, 2018). The most promising materials include genotypes of *S. chmielewskii*, *S. habrochaites* and *S. lycopersicum* × *S. peruvianum*. Also, an inbred line of tomato, LT05, has shown high tolerance to ToCV (González-Arcos *et al.*, 2018).

CONCLUSIONS AND PROSPECTS

In summary, ToCV can be considered a bona fide emergent plant virus that is still expanding its geographical and host ranges by means of its insect vectors. In addition, human-assisted movement of plant materials, planting monocultures or exotic crops, and introducing other agricultural practices may enhance virus spread. Getting a more precise knowledge of the mechanisms involved in its transmission by whiteflies, ongoing worldwide emergence, and disease development will guide future research efforts with the final goal of developing effective control measures. Some of the key research issues on which emphasis should be placed are listed below:

- The real molecular and biological diversity of ToCV should be revealed by sequencing and generation of infectious clones of isolates from plant hosts other than tomato.
- The molecular mechanisms underlying symptom development in plants infected by ToCV, an issue only barely studied (e.g. Seo *et al.*, 2018), should receive further attention in order to understand how the disease initiates and progresses in the plant after horizontal transmission by whiteflies.
- Almost nothing is known about the molecular determinants of whitefly transmissibility of ToCV, especially on the insect side, and this can be extended to other criniviruses and semipersistently transmitted plant viruses in general. Identification of virus receptors in the whitefly will be a key step to clarify the mechanism of transmission and to design strategies to disrupt disease spread.
- Establishing correlations between climatic variables and distribution of whiteflies of the *B. tabaci* complex [especially those shown to efficiently transmit ToCV: MED (formerly Q biotype), MEAM1 (formerly B biotype) and NW (formerly A biotype)], *T. vaporariorum* and *T. abutilonea* will help to predict areas where ToCV is likely to emerge. This takes on special importance from the perspective of climate change.
- The few known cases of synergisms in which ToCV is involved are insufficiently understood and should be further investigated, as should novel interactions that occur when the virus comes into contact with new viruses following its ongoing emergence.
- The development of resistant or tolerant commercial tomato (and other crop) varieties is urgently needed to complement basic and general control measures to reduce whitefly numbers and virus inoculum.

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