Detection of Tomato yellow leaf curl virus in imported tomato fruit in northern Europe

K. Justab, W. N. Lekebct, M. N. Sassarb, A. Luika and A. Kvarnhedenab*

*Department of Plant Protection, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, 51014 Tartu, Estonia; bDepartment of Plant Biology and Forest Genetics, Uppsala BioCenter, Swedish University of Agricultural Sciences, Linnean Center of Plant Biology, PO Box 7080, SE-750 07 Uppsala, Sweden; and cInstitute of Agricultural Research for Development (IRAD), Bambui, PO Box 80, Bamenda, Cameroon

Imported tomato fruits infected with Tomato yellow leaf curl virus (TYLCV) were identified on the market in northern Europe using paper-based FTA Classic Cards (Whatman), polymerase chain reaction (PCR) and partial DNA sequence analysis. Trade tomatoes originating from southern Europe, Africa and the Middle East were sampled in Estonia and Sweden, and tested for infection with begomoviruses. Out of 100 batches analysed with five fruits sampled in each batch (58 batches from Estonia and 42 from Sweden), 20 batches were positive (16 from Estonia and four from Sweden). Rolling circle amplification (RCA) and full-length genome sequence analysis of one isolate collected in Estonia and one isolate in Sweden, revealed highest nucleotide sequence identity at 99% to TYLCV-IL for the Estonian isolate and at 97% to TYLCV-Mld for the Swedish isolate. In this study, TYLCV was identified for the first time in imported tomato fruits on the market in northern Europe. FTA cards proved to be an effective means to collect, extract and store begomovirus DNA from tomato fruits and the subsequent molecular analysis.

Keywords: Begomovirus, FTA, Solanum lycopersicum, TYLCV

Introduction

Tomato (Solanum lycopersicum) is among the most important vegetable crops worldwide. It is susceptible to a large number of viral pathogens, among which begomoviruses (genus Begomovirus, family Geminiviridae), transmitted by whiteflies of the Bemisia tabaci species complex (order Hemiptera, family Aleyrodidae), are the most destructive, hampering the economic profitability of tomato production in various regions of the world (Hanssen et al., 2010). Apart from being a limiting factor for tomato growers in subtropical and tropical regions since the first disease outbreaks in Israel in 1939–1940, the begomovirus Tomato yellow leaf curl virus (TYLCV) has more recently appeared in temperate regions, causing concern for the production of tomato and other vegetables in greenhouses (Caciagli, 2007; Lefevre et al., 2010; Navas-Castillo et al., 2011). TYLCV may cause up to 100% yield loss and the induced symptoms include upward curling, size reduction and yellowing of leaves as well as flower abortion (Díaz-Pendón et al., 2010; Navas-Castillo et al., 2011).

The major factors in the worldwide spread of begomoviruses have been the emergence of the Middle East–Asia Minor 1 (MEAM1) species of the B. tabaci complex (formerly B biotype) with a wide host range and rapid expansion in geographic distribution (De Barro et al., 2011; Navas-Castillo et al., 2011), as well as evolution of virus variants through mutations, recombination and pseudorecombination. As with other infectious diseases of plants, anthropogenic activities are the main forces behind the distribution of begomovirus diseases: changes in cropping system, introduction of new crops and the trade of infected commercial as well as retail vegetable and ornamental plants, plant products, germplasm, grafts, cuttings and the vector (Polston et al., 1999; Hanssen et al., 2010; Navas-Castillo et al., 2011).

Begomoviruses are transmitted by B. tabaci in a circulative persistent manner and have twinned icosahedral particles (18 × 30 nm). The TYLCV genome consists of one component of circular single-stranded (ss) DNA with a size of 2.8 kb including six open reading frames (ORFs) and a noncoding intergenic region (IR) (Díaz-Pendón et al., 2010; Brown et al., 2012). On the virion (+) sense strand, there are two ORFs (V1 and V2) and on the complementary (−) sense strand, there are four ORFs (C1–C4) (Navot et al., 1991; Brown et al., 2012). V1 encodes a coat protein (CP), V2 a precoat or movement protein (MP), CI the replication initiator (Rep), C2

*E-mail: Anders.Kvarnheden@slu.se
†Present address: International Institute of Tropical Agriculture (IITA), PMB 5320, Oyo Road, 200001 Ibadan, Oyo State, Nigeria
‡Present address: Institute of Agricultural Sciences, University of the Punjab, PO Box 54590, Lahore, Pakistan

Published online 25 February 2014

© 2014 British Society for Plant Pathology
a transcriptional activator (TrAP), C3 a replication enhancer (REn) and C4 is a determinant of symptom expression (Brown et al., 2012; Fondong, 2013). The IR contains key elements for replication and transcription of the viral genome.

Tomato is affected by begomoviruses of more than 50 species, among which monopartite begomoviruses associated with tomato yellow leaf curl disease (TYLCD) are most destructive (Glick et al., 2009; Diaz-Pendón et al., 2010). The TYLCD complex includes c. 15 species (Navas-Castillo et al., 2011) and in Europe, four species are present: TYLCV, Tomato yellow leaf curl Axarquia virus (TYLCAxV), Tomato yellow leaf curl Malaga virus (TYLCMalV) and Tomato yellow leaf curl Sardinia virus (TYLCSV). TYLCV is the most dominant TYLCD-associated species and it is divided into different strains, among which the Israel (TYLCV-IL) and mild (TYLCV-Mld) strains are the most prevalent ones (Glick et al., 2009; Hanssen et al., 2010; Lefeuvre et al., 2010; Navas-Castillo et al., 2011). Isolates of these two strains do not induce significantly different symptoms, but they differ mainly in the nucleotide (nt) sequences of C1 (Rep protein). The genomes of both strains were cloned and sequenced in the 1990s (Navot et al., 1991; Antignus & Cohen, 1994) and the two strains are ranked among the greatest biotic threats to tomato production worldwide (Hanssen et al., 2010).

At present, control measures of TYLCV in trade tomato are focusing on the vegetative plant parts, mainly on imported tomato seedlings. Monitoring is not being applied to the imported and exported tomato fruit because it is not considered a potential risk as a virus source for B. tabaci. However, TYLCV is present at a high titre in tomato fruit and it can be acquired by B. tabaci and subsequently transmitted to healthy tomato plants (Delatte et al., 2003). Thus, not only plants and whitefly vectors can be a means of introduction of begomoviruses, but also the tomato fruit itself. In short, the spread of TYLCV through trade tomatoes should not be underestimated (Delatte et al., 2003). In addition, there are examples of other viruses introduced into new regions by traded fruit material. Melon has been reported as a means for long distance dissemination of cucurbit potyviruses (Lecoq et al., 2003) and stone fruit a pathway for the introduction of Plum pox virus (PPV, family Potyviridae) into a new region. As with B. tabaci, aphids can acquire PPV from peach fruit and transmit the virus to peach plants (Labonne & Quilot, 2001).

The aim of this project was to identify begomoviruses in imported tomato fruit, determine their incidence and to evaluate the use of FTA cards combined with polymerase chain reaction (PCR) and rolling circle amplification (RCA) as a means for collecting, and molecular analysis of, begomovirus infection in tomato fruit. In this study, the first TYLCV sequence information is provided for trade tomato fruits collected from the market in northern Europe, including two complete genome sequences. The nucleotide sequence data reported is available in the NCBI GenBank database under the accession numbers HF548825–HF548846.

### Materials and methods

#### Sample collection and preparation

During 2008–2009, a total of 100 batches of tomato fruits imported from southern Europe, Africa and the Middle East were collected in Estonia and Sweden (Table 1). Begomovirus-infected tomato fruit can be symptomless (Delatte et al., 2003), therefore the sampling was done randomly and sepalas were collected when available. Five fruits were sampled from each batch; therefore, 500 tomato fruits were tested in total. From each fresh fruit, five slices with a thickness of 0.2 cm and a diameter of 0.5 cm were cut using a sterile scalpel. A piece of Parafilm was placed over the fruit slices and a moderate pressure was applied with the round top of a plastic tube to press the sample onto an FTA card (Whatman) (Ndunguru et al., 2005). Cards were left to air dry overnight and they were stored at room temperature in paper bags and used for detection of begomoviruses by PCR or RCA.

#### Extraction of plant DNA from fresh tomato samples and FTA cards

To test if begomoviruses could be detected in tomato fruit by PCR, fruits, flowers or leaves from tomato plants infected with Tomato leaf curl Sinaloa virus (ToLCSinV) and Tomato severe leaf curl virus (ToSLCV) (Rojas et al., 2005) were homogenized in 10 mm Tris-HCl, pH 8.0. Fifty microlitres of the fruit homogenate was incubated overnight at 4°C in 0.2 mL PCR tubes. After removing the fruit extract, the PCR tubes were washed three times with 170 μL 10 mm Tris-HCl and used for PCR analysis. DNA was isolated from tissue of TYLCV-infected plants using Extract-N-Amp Plant PCR kit (Sigma-Aldrich) according to the manufacturer’s instructions.

DNA was also isolated from FTA cards using Extract-N-Amp Plant PCR kit (Sigma-Aldrich) according to the manufacturer’s instructions (Shepherd et al., 2008). In brief, three 2-mm diameter discs were punched from FTA cards using a Harris Uni-Core 2-00 mm puncher and a Harris Cutting Mat 2.5 × 3 mm (Whatman) and transferred to PCR tubes. Instead of 100 μl extraction solution as recommended by the manufacturer, 25 μl extraction solution was added and the tube was heated at 95°C for 10 min. Finally, 25 μl of dilution solution was added and the sample was stored at 4°C or used directly as template for PCR or RCA.

### Table 1. Detection of Tomato yellow leaf curl virus (TYLCV) in 100 batches of tomato fruit (five fruit per batch) imported into Estonia and Sweden during 2008–2009

<table>
<thead>
<tr>
<th>Origin</th>
<th>Estonia</th>
<th>Sweden</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canary Islands</td>
<td>0/1</td>
<td>0/2</td>
</tr>
<tr>
<td>Egypt</td>
<td>0/5</td>
<td>1/7</td>
</tr>
<tr>
<td>France</td>
<td>0/1</td>
<td>0/0</td>
</tr>
<tr>
<td>Israel</td>
<td>0/5</td>
<td>1/10</td>
</tr>
<tr>
<td>Italy</td>
<td>0/0</td>
<td>1/3</td>
</tr>
<tr>
<td>Morocco</td>
<td>1/8</td>
<td>0/4</td>
</tr>
<tr>
<td>Netherlands</td>
<td>0/3</td>
<td>0/0</td>
</tr>
<tr>
<td>Senegal</td>
<td>0/2</td>
<td>0/3</td>
</tr>
<tr>
<td>Spain</td>
<td>15/32</td>
<td>1/13</td>
</tr>
</tbody>
</table>
Detection of TYLCV from tomato fruits using PCR

For initial TYLCV detection, the degenerate PCR primers AV494 and AC1048 were used to amplify a c. 0.6 kb fragment of the begomoviral CP core region of the V1 gene (Wyatt & Brown, 1996) as previously described (Leke et al., 2012). Tomato samples infected with ToLCsInV and ToSLCV were used as positive controls (Rojas et al., 2005). PCR mixtures including water or extract from empty FTA cards were used as negative controls. The PCR amplification product was purified using a PCR Purification kit (Fermentas), cloned into pGEM-T Easy (Promega), and transformed into Escherichia coli DH5α subcloning-efficiency competent cells (Invitrogen). Three clones of each isolate were sequenced in the forward direction and one clone in both directions with M13 forward and reverse primers using ABI 3730xl or ABI 3700 DNA sequencers (Macrogen Inc.).

Full-length genome amplification of TYLCV DNA from tomato fruits

Amplification of begomoviral circular DNA was performed by RCA using the TempliPhi kit (GE Healthcare) following the manufacturer’s protocol. In brief, 2 µl extracted DNA was mixed with 5 µl sample buffer, denatured for 3 min at 95°C and cooled down on ice. After adding 5 µl reaction buffer and 0.2 µl enzyme mix, the reaction was incubated for 18 h at 30°C and stopped by incubation for 10 min at 65°C. For cloning, the RCA products were digested with different restriction endonucleases: the single-cutting enzymes SacI and NcoI were chosen for TYLCV isolates from Estonia and Sweden, respectively.

Cloning and sequencing of full-length viral genomes

The digested RCA products (c. 2.8 kb) were purified using GeneJET PCR purification kit (Fermentas). Purified DNA of the TYLCV isolate from Estonia was inserted into SacI-digested pGEM-3Zf(+) (Promega) and named pGTYE-1.0. Viral DNA of the isolate from Sweden was inserted into Ncol-digested pGEM-5Zf(+) (Promega) and named pGT YE9-1.0. Sequencing was done by Macrogen Inc. using M13 forward and reverse primers as well as sequence-specific primers.

Sequence analysis

To obtain provisional virus identification, the cloned VI PCR fragments were compared with known TYLCV sequences through searches of GenBank using BLASTN (Altschul et al., 1990). Identified full-length clones were compared with published full-length sequences of TYLCD-associated viruses. Alignments and phylogenetic analyses were performed with GENEIOUS (Drummond et al., 2012) using the Jukes–Cantor genetic distance model, neighbour joining and bootstrapping with 1000 replicates and 70% support threshold.

Results

Amplification of TYLCV VI gene from tomato fruits and sequence analysis

When testing the suitability of tomato fruit material as a source for PCR, fresh tomato fruit samples from plants infected with the New World bipartite begomoviruses ToLCSInV and ToSLCV (Rojas et al., 2005) gave the expected amplification product of 0.6 kb (results not shown). Begomovirus DNA was also amplified from fresh flower bud, flower and sepal samples from the same plants. Three samples of flower buds, five flowers, 10 fruits and eight sepals were tested and PCR was positive for all of them. Fruit pericarp was also tested separately from the mesocarp, resulting in equal efficiency of PCR amplification. DNA extraction from tomato tissue, directly or after sampling on FTA cards, followed by PCR was found to be effective for testing of begomovirus infection (Fig. 1). To facilitate the sampling, FTA cards were then used to monitor the presence of begomoviruses in imported tomato fruit in Estonia and Sweden. For the survey, 100 batches, with 500 fruits sampled in total, were collected on FTA cards (each FTA sample comprising five tomato fruits) and tested for begomovirus infection by PCR. Out of these, 20 samples were positive, producing a PCR product of the expected size (579 bp, including primers). Sixteen of these samples were collected in Estonia and four samples in Sweden (Table 1), and they originated from five countries in the Mediterranean region.

The authentication of the amplified PCR products as a DNA fragment of the begomovirus VI gene was performed by cloning and sequencing. Sequence comparisons showed that PCR fragments of the 16 positive tomato fruit samples collected in Estonia showed 98–100% nucleotide sequence identity to TYLCV isolates from Morocco (accession number EF060196), Spain (AJ489258) and the Netherlands (FJ439569). The VI sequences of the four positive samples from Sweden were 98–99% identical to TYLCV isolates from Morocco (EF060196), Spain (AJ489258), the Netherlands (FJ439569), Tunisia (EF101929), Italy (DQ144621), Egypt (EF107520) and Jordan (GQ861426). A phylogenetic analysis with partial nucleotide sequences (core CP region) supported the identification of TYLCV in imported fruit (Fig. 2). However, with the analysed genome region it was not possible to separate the different TYLCV strains (Lefeuvre et al., 2010).

![Figure 1 Detection of Tomato yellow leaf curl virus in tomato fruits and leaves by PCR. The results of the PCR analysis were visualized using gel electrophoresis. Lane M, 1 kb marker; lane 1, empty; lane 2, DNA extracted from fruit pressed onto FTA card; lane 3, DNA extracted from fresh fruit; lane 4, DNA extracted from leaves pressed onto FTA card; lane 5, DNA extracted from fresh leaves; lane 6, empty; lane 7, negative FTA control; lane 8, negative PCR control (H2O).](image-url)
Amplification and sequence analysis of full-length TYLCV genomes

To obtain conclusive virus identification, the genomes for one TYLCV isolate each from Estonia and Sweden were completely sequenced. The length for the full-length genomes was 2787 and 2798 nt for the isolates from Estonia and Sweden, respectively. In both genomes, the six expected ORFs were found as well as an IR (Brown et al., 2012).

The complete genome of the TYLCV isolate from Estonia (TYLCV-IL[EE:Imp:5:08]) showed the highest nucleotide identity, 99%, to TYLCV-IL[ES:Alm:Pep:99] (AJ489258) and a TYLCV-IL isolate from Mauritius (HM448447), and 97–98% identity to isolates from China, Jordan, Morocco, the Netherlands and many other regions. The nt identity to isolates of the mild strain of TYLCV was 93% or lower. The complete genome of the Swedish isolate (TYLCV-Mld[SE:Imp:2:09]) showed the highest identity, 98%, to TYLCV-Mld[JO:Hom:03] (AY594175) and at 97% to TYLCV-Mld isolates from Jordan (EF158004), Portugal (AF105975), Spain (AF071228) and Réunion (AJ865337). The nucleotide identity to isolates of the Israel strain was 94% or lower. The two full-length genomes sequenced in this study were 92% identical to each other. As with other TYLCV-IL isolates, TYLCV-IL[EE:Imp:5:08] differed in sequence from TYLCV-Mld[SE:Imp:2:09] in the IR, C1 and C4 regions (Navas-Castillo et al., 2000) while the nucleotide sequences of V1, V2, C2 and C3 of the two isolates were highly identical (97–99%).

A phylogenetic analysis was carried out for the sequenced complete genomes, including representative full-length sequences of different TYLCV strains and other tomato-infecting begomoviruses (Fig. 3). Sequences of the mild and Israel strains formed two separate clades, with the Estonian isolate in the clade of the TYLCV-IL strain (bootstrap value 100%) and the Swedish isolate in the clade of the TYLCV-Mld strain (bootstrap value 100%). The phylogenetic analysis confirmed that the two isolates collected from Estonia and Sweden belonged to different strains of TYLCV.

Discussion

Viruses are transported in the phloem together with carbohydrates from source to sink tissues. The movement of TYLCV follows this pattern (Wege, 2007) and the virus has been shown to accumulate in sink tissues such as roots, shoots, flowers and fruit (Navot et al., 1989; Ber et al., 1990) with high titres in the fruit (Delatte et al., 2003). The current results confirm that both bipartite (ToLCSinV and ToSLCV) and monopartite begomoviruses (TYLCV) accumulate and can be detected in tomato fruit.

Figure 2  Neighbour-joining analysis showing predicted relationships between tomato yellow leaf curl disease-causing begomovirus isolates based on partial V1 nucleotide sequences (536 bp, core region of coat protein). Tomato mottle virus (ToMoV) DNA-A was used as an out-group. Isolates sequenced in this study are indicated in bold. Sequences for Estonian TYLCV isolates 2, 3, 7 and 10 were identical and only the sequence of isolate 2 was included in the phylogenetic analysis. Horizontal lines are in proportion to the number of nucleotide substitutions per site. The bar represents nucleotide substitutions per site. Numbers represent percentages of bootstrap replicates that support each node (1000 replicates). Only bootstrap values higher than 70% are shown. Abbreviations are according to Brown et al. (2012).
TYLCV is in the EPPO (European and Mediterranean Plant Protection organization) A2 list of quarantine pests and all plant material that could carry *B. tabaci*-transmitted viruses is subjected to quarantine control (Anon, 2000). In control screens, TYLCD-associated viruses and *B. tabaci* are regularly intercepted in new regions (Caciglì, 2007; Powell *et al.*, 2012). Thus, the threat of TYLCD to become widespread in Europe is a reality with the movement of TYLCV-infected tomato material and appearance of *B. tabaci* in the fields and greenhouses of southern Europe, and in greenhouses of more northern areas (Picó *et al.*, 1996; Briddon, 2002; Powell *et al.*, 2012). For example, in 2007, a TYLCV outbreak in the western part of the Netherlands was reported in 20 neighbouring tomato-producing greenhouses; the outbreak resulted from a single introduction of the virus and *B. tabaci* further accounted for the local spread (Botermans *et al.*, 2009). It is expected that climate change will further enhance the spread of the virus and vector to previously unfavourable regions (Rodoni, 2009; Hannsen *et al.*, 2010; Navas-Castillo *et al.*, 2011). The problem is further complicated because TYLCV-infected fruit is symptomless (Picó *et al.*, 1996). Therefore, TYLCV in imported tomato fruits may possibly enable invasion of begomoviruses into new regions (Delatte *et al.*, 2003; Wege, 2007). In a survey carried out on Réunion Island, 13 of 27 batches of imported tomato fruit from Mediterranean countries were found to be infected with TYLCV (Delatte *et al.*, 2003) and the present study demonstrated that 20% of the tested imported tomato batches were positive for the same begomovirus. Sequence analysis of cloned PCR and RCA products revealed infection with isolates of the two common strains TYLCV-IL and TYLCV-Mld in the fruit samples. The incidence of begomoviruses in the analysed fruit samples may be an underestimate because the primers used in the survey may fail to detect some begomoviruses, such as TYLCSV (Accotto *et al.*, 2000).

FTA cards have been shown to be a practical, economical and sensitive method for sampling, storage and molecular testing of both RNA and DNA viruses from tissues of different plants (Ndunguru *et al.*, 2005; Roy & Nassuth, 2005; Guenoune-Gelbart *et al.*, 2010). The combination of FTA cards and RCA was also shown to be efficient for large-scale field surveys when analysing maize leaf samples for infection with the mastrevirus (family Geminiviridae) *Maize streak virus* (MSV; Owor *et al.*, 2007). Compared to PCR, RCA has the advantage that it can be used to amplify full-length viral genomes without the need for specific primers (Haible *et al.*, 2006). The combination of FTA/PCR for the testing of tomato fruit samples and FTA/RCA was successfully applied in the current study to obtain full-length TYLCV genomes. The Extract-N-Amp Plant PCR kit was used to extract DNA from FTA cards and the combination of Extract-N-Amp/FTA/RCA was found to be an efficient
method for extraction, amplification and cloning of full
length begomivirus genomes. Evidence is provided here
for the presence of TYLCV in imported tomatoes on the
market in northern Europe, reporting the first se-
quence identification of TYLCV from imported tomatoes
in Estonia and Sweden.

Acknowledgements

The authors wish to thank Nina Lukhovitskaya for pro-
viding cloning vector pGEM-5Zf(+) and advice. Also, they
thank Usman Arif and Ingrid Eriksson for technical assis-
tance and helpful discussions. This research was sup-
ported by the European Social Fund’s Doctoral Studies
and Internationalisation Programme DoRaK, the Estonian
Ministry of Education, and the Archimedes Foundation.
W. N. L. was supported by Rothamsted International
through the Rothamsted International African Fellows
Programme and by The Swedish Institute (SI). M. N. S.
was supported by the HEC/Swedish Institute under the
‘Overseas Scholarship Scheme for PhD in Selected Fields’.

References

Typing of Tomato yellow leaf curl viruses in Europe. European
Journal of Plant Pathology 106, 179–86.
local alignment search tool. Journal of Molecular Biology 215,
403–10.
measures against the introduction into the Community of organisms
harmful to plants or plant products and against their spread within the
169, 1–112.
infectious clone of a mild isolate of Tomato yellow leaf curl virus
(TYLCSV). Phytopathology 84, 707–12.
Infection of tomato by the Tomato yellow leaf curl virus: susceptibility
to infection, symptom development, and accumulation of viral DNA.
Botermans M, Verhoeven TJT, Jansen CCC, Roenhorst JW, Striger
CCMM, Pham KTK, 2009. First report of Tomato yellow leaf curl
virus in tomato in the Netherlands. Plant Disease 93, 1073.
Brown JK, Fauquet CM, Bridgon RW, Zerbini M, Moriones E,
MJ, Carstens EB, Lefkowitz EJ, eds. Virus Taxonomy: Classification
and Nomenclature of Viruses - Ninth Report of the International
Committee on Taxonomy of Viruses. Oxford, UK: Elsevier Academic
Press, 351–73.
transportation of agricultural products and plants. In: Czosnek H, ed.
Tomato Yellow Leaf Curl Virus Disease. Dordrecht, The Netherlands:
Springer, 57–63.
Dellate H, Dalmon A, Rist D et al., 2003. Tomato yellow leaf curl virus
can be acquired and transmitted by Bemisia tabaci (Gennadius) from
tomato fruit. Plant Disease 87, 1297–300.
Diaz-Pendón JA, Canizares MC, Moriones E, Bejarano ER, Czosnek H,
trois between the virus complex, the plant and the whitefly vector.
Molecular Plant Pathology 11, 441–50.
Drummond AJ, Ashton B, Buxton S et al., 2012. GENEIOUS v. 5.6. [http://
Molecular Plant Pathology 14, 635–49.
Glick E, Levy Y, Gafni Y, 2009. The viral etiology of tomato yellow leaf
Guennoue-Gilbert D, Sufirin-Ringwald T, Capobianco H, Gaba V,
Polston JE, Lapidot M, 2010. Inoculation of plants with
begomoviruses by particle bombardment without cloning: using rolling
circle amplification of total DNA from infected plants and whiteflies.
Haible D, Kober S, Jeske H, 2006. Rolling circle amplification
revolutionizes diagnosis and genomics of geminiviruses. Journal of
Virological Methods 135, 9–16.
Labonne G, Quiot JB, 2001. Aphiids can acquire Plum pox virus from
involvement of melon fruit in the long distance dissemination of
Lefèvre P, Martin DP, Harkins G et al., 2010. The spread of Tomato
yellow leaf curl virus from the Middle East to the world. PLoS
Pathogens 6, e1001164.
Leke WN, Brown JK, Lighart ME, Sattar N, Niaulem DK, Kvarnheden
A, 2012. Ageratum conyzoides: a host to a unique begomovirus
Navas-Castillo J, Sánchez-Campos S, Noris E, Lourido D, Accotto GP,
Moriones E, 2000. Natural recombination between Tomato yellow
leaf curl virus-Is and Tomato leaf curl virus. Journal of General
Virology 81, 2797–801.
virus diseases transmitted by whiteflies. Annual Review of
curl virus in squashes of plants and insect vectors. Phytopathology
yellow leaf curl virus: a whitefly-transmitted geminivirus with a single
Nduguuru J, Taylor NJ, Yadav J et al., 2005. Application of FTA
technology for sampling, recovery and molecular characterization of
viral pathogens and virus-derived transgenes from plant tissues.
Virology Journal 2, 45.
Oower BE, Shepherd DN, Taylor NJ et al., 2007. Successful application
of FTA® Classic Card technology and use of bacteriophage q29
DNA polymerase for large-scale field sampling and cloning of
complete maize streak virus genomes. Journal of Virological Methods
140, 100–5.
economic losses to the tomato crop. II. The Tomato yellow leaf curl
yellow leaf curl virus in Florida and implications for the spread
of this and other geminiviruses of tomato. Plant Disease 83,
984–8.
Powell ME, Cuthbertson AGS, Bell HA, Boonham N, Morris J, North
P, 2012. First record of the Q Biotype of the sweetpotato whitefly,
Bemisia tabaci, intercepted in the UK. European Journal of Plant
Pathology 133, 797–801.
Rodini B, 2009. The role of plant biosecurity in preventing and controlling
characterization of Tomato leaf curl Sinaloa virus and Tomato severe
leaf curl virus: phylogeny of New World begomoviruses and detection


---

**A Note from the Editor**

Matt Dickinson

We would like to take this opportunity to acknowledge the hard work of all our Editorial Board members and also the efforts of all the anonymous reviewers that we have used during the past year. We thank them all for their invaluable contributions, and for helping to maintain the standards and continued success of the Journal.

Doi: 10.1111/ppa.12276