Population shifts in begomoviruses associated with tomato yellow leaf curl disease in western Mediterranean countries

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Abstract

Tomato yellow leaf curl disease (TYLCD) was reported in western Mediterranean basin since the late 1980s. Based on intensive plant samplings performed in Spain, Italy and Morocco at different periods between the 1990s and 2014, several begomoviruses (family Geminiviridae) were identified as the cause of TYLCD. They comprise the native Tomato yellow leaf curl Sardinia virus (TYLCSV), two strains of Tomato yellow leaf curl virus (TYLCV) introduced from the Middle East, and several types of TYLCV/TYLCSV recombinants including the invasive recombinant TYLCV-IS76 in which the proportion of genome inherited from TYLCSV was unusually short. Although parental and recombinant TYLCD-associated begomoviruses were present in each country, country specificities were detected with respect to their relative prevalence, the infection profiles of individual tomato plants, and the recombination patterns of TYLCV/TYLCSV recombinants. Considering geographic proximities and trade activities between these countries, and the efficient transmission of begomoviruses which are persistently transmitted by the polyphagous and tiny whitefly vector *Bemisia tabaci*, it was not known if these specificities would be maintained over time. To address this question, tomato samples collected in the three countries between 2015 and 2019 were analysed with PCR tests previously designed to distinguish species, strains and recombinants of TYLCD associated begomoviruses, and with an original PCR test distinguishing emerging resistance-breaking recombinants bearing short TYLCSV-inherited fragments (Srec) from formerly reported recombinants with longer TYLCSV fragments (Lrec). The results show that country specificities are still present, the most striking being the contrasted geographic distribution of Srec recombinants, with TYLCV-IS76 detected in Morocco but not in Italy, and TYLCV-IS141 and a new Srec recombinant (TYLCV-IS60) both detected in Italy and not in Morocco. Nevertheless, besides country specificities, a general population shift was revealed regarding TYLCV/TYLCSV recombinants. Indeed, all the recombinant positive samples, irrespective of their geographic origin, were Srec-positive but Lrec-negative, which suggest that the emergence of Srec recombinants occurred at the expenses of the Lrec recombinants. These results are discussed in relation to the positive selection of Srec recombinants by Ty-1 resistant tomato plants.

1. Introduction

Tomato (*Solanum lycopersicum*) is one of the New World (NW) originated vegetables that is presently grown worldwide including Mediterranean countries. During its global spread, it encountered Old World (OW) viruses and particularly viruses belonging to the genus *Begomovirus* of the family *Geminiviridae*. Indeed, according to the International Committee on Taxonomy of Viruses (https://ictv.global/taxonomy), 112 Begomoviruses were isolated from tomato worldwide and most of them (69) from the OW. Noteworthy, a typical feature of OW begomoviruses is their ability to induce leaf curl, a feature which is highlighted in all the 69 names, as for example Tomato yellow **leaf curl**

virus (TYLCV) or Tomato yellow **leaf curl** Sardinia virus (TYLCSV). The symptoms induced by the 43 NW begomoviruses are much more diverse and only 8 of them were characterized by the terms “leaf curl” in their names.

Following the introduction of tomato in the western Mediterranean region, it encountered at least two OW begomoviruses, TYLCSV and TYLCV. They both cause tomato yellow leaf curl disease (TYLCD), an economically important disease of tomato. TYLCSV was reported first (Kheyr-Pour et al., 1991; Luisoni et al., 1989; Noris et al., 1994) and is generally considered to be indigenous (Lefeuvre et al., 2010). TYLCV is a foreign begomovirus most probably from eastern Mediterranean countries (Lefeuvre et al., 2010). Two TYLCV strains were reported from western Mediterranean countries, TYLCV-Israel (TYLCV-IL) and the so-called mild strain, TYLCV-Mld. In Spain, the earliest plant samples in which TYLCV-Mld was detected were collected in 1997 (NavasCastillo et al., 1997) and TYLCV-IL, in 2002 (Morilla et al., 2003). In Italy, the earliest collected samples in which TYLCV was detected are from 2002 for the IL strain (Accotto et al., 2003) and 2012 for the Mld strain (Belabess et al., 2015). In Morocco, the earliest plant samples in which TYLCV-IL and TYLCV-Mld were detected, were collected in 1998 (Belabess et al., 2015; Peterschmitt et al., 1999). Although all three countries host TYLCD-associated viruses of the same species, country specificities were detected. For example, the displacement of TYLCSV induced by the arrival of TYLCV was different between Spain and Italy. Indeed, whereas in Spain the displacement was virtually total, in Italy, virus populations reached an equilibrium in which the two virus species coexist (Davino et al., 2006; Davino et al., 2009; Garcia-Andres et al., 2007; SanchezCampos et al., 1999).

Since the spread of TYLCV into western Mediterranean countries, the diversity of begomoviruses has increased considerably, and not only because of representatives of a new virus species, i.e. *Tomato leaf curl New Delhi virus* (ToLCNDV) (Juárez et al., 2013), but also due to recombinants between TYLCV and TYLCSV as reported from Spain (Garcia-Andres et al., 2006; Monci et al., 2002), Italy (Belabess et al., 2015; Davino et al., 2008; Granier et al., 2019; Panno et al., 2018) and Morocco (Belabess et al., 2015).

The earliest collection of plant samples detected positive for TYLCV/TYLCSV recombinants is in 1998 in Spain and Morocco (Belabess et al., 2015; Monci et al., 2002) and 2002 in Italy (Davino et al., 2008). Although the collection dates of these recombinant positive samples were fairly similar, the earliest dates at which plants were detected with only recombinants, i.e. testing negative for parental viruses, are very different. Indeed, whereas in Spain such plants were detected 1 year after the first detection of recombinants (Monci et al., 2002), in Morocco and Italy the delay was at least 10 years (Belabess et al., 2015). In natural conditions, when a recombinant is detected in a plant without parental viruses, it generally means that it is fit enough to spread autonomously from plant to plant and is potentially emerging. It seems therefore that the agroecological environment of Spain was more conducive to the emergence and spread of TYLCV/TYLCSV recombinants than the Italian and Moroccan environment. The contrasted practice of bean growing between Southern Spain and Sicily was suggested to be critical (Davino et al., 2009); in Southern Spain, beans (Phaseolus vulgaris) are regularly grown between tomato crops, but not in Sicily. Thus, this specific growing practice of beans may have favoured the emergence of the bean adapted recombinant Tomato yellow leaf curl Malaga virus (TYLCMalV) in Spain (Monci et al., 2002).

Another country specificity is related to the recombination profiles of the autonomously spreading TYLCV/TYLCSV recombinants. The two spreading recombinants reported from Spain, i.e., TYLCMalV (Monci et al., 2002) and Tomato yellow leaf curl Axarquia virus (TYLCAxV) (Garcia-Andres et al., 2006) have both inherited a long TYLCSV fragment of at least 1000 nts, including at least the two viral strand-encoded genes V1 and V2 and their promoter region up to the origin of replication. Although this type of recombinants were also reported from Italy (Davino et al., 2012) and are suspected from Morocco (Belabess et al., 2015), they were not found to spread autonomously. Unlike in Spain, the spreading recombinants that emerged in Italy and Morocco inherited a much shorter TYLCSV fragment, located

between the origin of replication and the 5' end of the V2 gene. Intriguingly, while the emerging recombinants reported from Italy have a TYLCSV-inherited fragment of 141 nts (Belabess et al., 2015; Granier et al., 2019; Panno et al., 2018), the emerging recombinant reported from Morocco has only a 76 nts fragment inherited from TYLCSV (Belabess et al., 2015). These recombinants were named TYLCV-IS76 and TYLCV-IS141 according to the position of their typical recombination breakpoints (Belabess et al., 2015; Belabess et al., 2018). Noteworthy, TYLCV-IS76 recombinants were recently detected in Spain (Fortes et al., 2023; Torre et al., 2019) and due to their high nucleotide identity with Moroccan representatives, they presumably originate from Morocco. Noteworthy, TYLCV-IS76 and TYLCV-IS141 exhibit resistance breaking features. Indeed, they were both detected on symptomatic tomato plants bearing the Ty-1 resistance gene (Belabess et al., 2015; Panno et al., 2018).

Considering geographic proximity and trade activities between Morocco, Italy and Spain, and the efficient and persistent transmission of these viruses with the polyphagous winged and tiny whitefly vector *Bemisia tabaci*, it was not known if these specificities would be maintained or rather fade over time due to natural or anthropogenic-induced virus spread. To address this question, we analysed the infection profiles of tomato samples collected from the three countries between 2015 and 2019. The infection profile of each plant was first determined with the multiplex PCR tests described by Belabess et al (Belabess et al., 2015). Then, to monitor more specifically the prevalence of recombinants with a resistance breaking potential like TYLCV-IS76 and TYLCV-IS141, a new test was designed to distinguish such recombinants characterized by their short TYLCSV inherited fragment (Srec), from those that inherited longer TYLCSV fragments like TYLCMalV and TYLCAxV (Lrec). Although the results show that country specificities are maintained with contrasted prevalence of recombinant and parental type viruses between countries, they also show a general population shift with the emergence of Srec recombinants at the expenses of the Lrec recombinants which were not detected in any of the samples from the three countries.

2. Material and Methods

2.1. Plant material

Leaf samples were collected from protected tomato crops between 2015 and 2019 in Morocco, Spain and Italy.

In Morocco, 3 samples were collected in December 2015 in Agadir, and 48 samples were collected in 2017 from 6 locations along the Atlantic coast between Casablanca and Agadir. These 48 samples were from 12 farms, with 4 samples per farm collected on tomato plants exhibiting TYLCD symptoms. Collection sites from North to South were Azmour with 1 farm sampled in April, Chtouka ouled laissaoui with one farm sampled in March, Ouled Frej with 3 farms sampled in February, Oualidia with 4 farms sampled in March, Safi doukkala had lhrara with one farm sampled in March, and Agadir with 2 farms sampled in January.

In Italy, 37 samples were collected between 2015 and 2019. Four samples are from Sardinia and 33 samples from Sicily. Samples from Sardinia were previously reported (Granier et al. 2019) but were further analysed here with a PCR test distinguishing Lrec and Srec type recombinants.

In Spain, a total of 17 samples were collected between 2015 and 2018 in the South-East of the country within the tomato-growing regions of Murcia and Almeria

2.2. DNA extraction

The 48 leaf samples collected in 2017 in Morocco were lyophilized and total DNA was extracted with a reported protocol (Dellaporta et al., 1983) modified as follows. The amount of lyophilized plant material used per extraction ranged between 1.25 and 20 mg. Plant material was ground in 400 µL extraction buffer (100 mM Tris-HCl pH 8.0, 50 mM EDTA, 500 mM NaCl, 1% SDS, 36 mM Na_2SO_3, and

141 0.1 mg/ml RNase), incubated at 65°C for 10 min and centrifuged (16,000 g, 10 min). One volume of
142 isopropanol was added to 300 µL of the supernatant and nucleic acids were precipitated by
143 centrifugation (16,000 g, 20 min); the pellet was washed with ethanol 70% and then resuspended in
144 50 µL water. As no PCR product was detected with 32 samples, a second DNA extraction was done with
145 a silica-based DNA extraction kit (DNeasy Plant Mini Kit, QIAGEN S.A., Courtaboeuf, France) with 10
146 mg of lyophilized plant material per extraction.

147 Total DNA from all the other samples from Morocco, and those from Italy and Spain was extracted
148 with the silica-based DNA extraction kit "NucleoSpin Plant II, Mini kit for DNA from plants" (Macherey
149 Nagel SAS, Hoerdt, France), except for the samples from Sardinia for which the Qiagen-DNeasy Plant
150 Mini kit was used.

151 2.3. PCR tests and primer design

152 Plant samples were first analysed with the two multiplex PCR tests MxPCR1 and MxPCR2, with which
153 the presence of TYLCV-IL, TYLCV-Mld, TYLCSV, and recombinants derived from them (Belabess et al.,
154 2015) can be specifically detected. Due to the fact that all reported TYLCV/TYLCSV recombinants bear
155 a recombination breakpoint at the origin of replication (OR), the test is expected to detect all
156 recombinants between TYLCV-IL and TYLCSV and between TYLCV-Mld and TYLCSV. Another typical
157 feature of these reported recombinants is that the sequence contiguous to OR and located at the
158 complementary sense gene side was always inherited from TYLCV, whereas the sequence located at
159 the other side is always inherited from TYLCSV; reciprocal recombinants have not been reported so
160 far.

161 Samples detected positive for the presence of TYLCV/TYLCSV recombinants with MxPCR1, were
162 analysed further with original PCR tests designed in this study to distinguish between recombinants
163 that inherited a short TYLCSV fragment (Srec) like TYLCV-IS76 or TYLCV-IS141, from those that inherited
164 long TYLCSV fragments (Lrec) like TYLCAxV (Garcia-Andres et al., 2006) or TYLCMalV (Monci et al.,
165 2002). The test is described below in the section "Results".

166 2.4. Sequencing of PCR products and sequence analysis

167 Some of the samples in which Srec type recombinants were detected by PCR, were further analysed
168 by sequencing to determine non-OR recombination breakpoints. To do this, the PCR products
169 generated with Srec-specific PCR primers were sequenced by Sanger sequencing (Eurofins Genomics,
170 Ebersberg, Germany) using the PCR primer IM650R, complementary to the viral sense strand of the
171 viral genome (Fig. 1A, Table 1).

172 The samples from Spain in which TYLCV was detected were further analysed by sequencing to test if
173 the detected TYLCV isolates may belong to any of the two groups that were previously identified by
174 sequence comparisons of viruses isolated from tomato plants sampled in Southeast Spain in 2015 and
175 2016 (Torre et al., 2018). These authors distinguished two TYLCV groups, group 1 and group 2. Group
176 1 viruses are related to the two early TYLCV-IL isolates of Southeast Spain, both sampled on pepper
177 (*Capsicum annuum*), one in 1999 in Almeria (TYLCV-IL[ES:Alm:Pep:99], GenBank accession number
178 AJ489258) (Morilla et al., 2005) and one in 2003 in Malaga (TYLCV-IL [ES:Mlg:TY11:Pep2003], GenBank
179 accession number KC953602). Group 2 viruses are closely related to two TYLCV-IL isolates sampled on
180 tomato in Morocco, TYLCV-IL[MA:El Jadida:Tom:2013] (GenBank accession number LN846613 and
181 LN846613) (Belabess et al., 2015). To test the relatedness of TYLCV isolates with viruses of group 1 or
182 2, we PCR-amplified and sequenced a DNA fragment of the TYLCV genomes. The fragment was
183 amplified with the forward primer IL-2629F (5'-GGTGTCCCTCAAAGCTCTAWG-3') of the multiplex PCR
184 test MxPCR2 (Belabess et al., 2015) in association with the reverse primer IM650R also used for Sanger-
185 sequencing (Table 1). The region amplified by PCR was selected because of its relatively high density
186 of group1/group2 discriminating nucleotides, with 11 positions within a 592-nucleotide fragment of
187 the PCR product. Sequences were aligned with homologous genomic sequences from TYLCVs isolated

188 in Spain and for which complete viral genomes are available in Genbank. The alignment was performed
189 with the Optimal Alignment method of DNAMAN (version 5.0; Lynnon BioSoft, Quebec, Canada). A
190 phylogenetic tree was set up with a Jukes and Cantor distance matrix using the neighbour-joining
191 method (Saitou and Nei, 1987) as implemented in the DNAMAN software.

192 2.5. Cloning and sequencing of complete viral genomes

193 A new Srec type recombinant bearing a recombination breakpoint at position 60 was detected by
194 sequencing PCR products. To characterize the full recombination profile of this new type of Srec
195 recombinants, their complete genome was cloned from total DNA extracted from three plant samples
196 with the DNeasy Plant Minikit. Circular DNA molecules were amplified using a TempliPhi Amplification
197 kit (GE Healthcare, UK) according to the manufacturer's instructions, and digested with restriction
198 enzymes that are expected to be potentially unique according to reported sequences of parental type
199 genomes of TYLCV and TYLCSV. Digestion of RCA products with SacI generated linear fragment of about
200 2.8kb, a size expected with full-length begomovirus genomes. The restricted fragments were ligated
201 into the plasmid pBC and ligation products were introduced into the bacterial strain *Escherichia coli*
202 DH5alpha. The cloned fragments were sequenced with M13 primers and virus-specific primers.
203 Sequences were analysed with Chromas (Technelysium Pty Ltd, Australia) and DNAMAN.

204

205 **3. Results**

206 3.1. Srec and Lrec recombinants can be distinguished with a discriminating PCR test

207 An original PCR test was designed to distinguish TYLCV/TYLCSV recombinants that inherited short
208 TYLCSV derived fragments of less than 623 nts (Srec), from those that inherited TYLCSV-derived
209 fragments of more than 623nts (Lrec).

210 The pair of PCR primers targeting Srec recombinants, comprise the forward primer Sar19F,
211 complementary to TYLCSV genome sequences, and the reverse primer IM650R, complementary to
212 TYLCV genome sequences (Fig 1A and Table 1). The reverse primers Ty153R and Ty282R were used to
213 further analyse Srec-positive samples for the presence of IS76- or IS141-type recombinants. The
214 forward PCR primer targeting Lrec recombinants is Sar599F, a primer complementary to TYLCSV
215 genome sequences (Fig 1C and Table 1). To distinguish Lrec having a TYLCV-IL parental virus like
216 TYLCAxV (Garcia-Andres et al., 2006) from Lrec having a TYLCV-Mld parental virus like TYLCMalV
217 (Monci et al., 2002), two reverse primers were used, IL-2258R and Mld-2310R, respectively.
218 Noteworthy, as the TYLCV-IL and TYLCV-Mld genomes can be distinguished only between position 2000
219 and the end of the genome, it was not possible to infer from the Srec PCR products, located between
220 position 1 and 650, whether the TYLCV parent of the Srec was of the IL or the Mld strain. Specificity of
221 PCR primers were determined in-silico with a range of TYLCV-IL, TYLCV-Mld and TYLCSV genomes
222 available in GenBank. Some of the primers were degenerated according to the intra-species nucleotide
223 diversity of reported genomes.

224 The cycling protocol for the amplification of Lrec recombinants was as follow: 5 min denaturation at
225 95 °C, followed by 30 cycles, each consisting of 45 s at 95 °C, 45 s at 50 °C, and 90 s at 72 °C. The PCR
226 program was ended by a 10 min incubation at 72 °C. The same protocol was used for Lrec-IL and Lrec-
227 Mld. The PCR products were resolved by electrophoresis in a 1% agarose gel and visualized with
228 ethidium bromide staining.

229 The cycling protocol for the amplification of Srec recombinants was as follows: 5 min denaturation at
230 95 °C, followed by 30 cycles, each consisting of 45 s at 95 °C, 45 s at - 52 °C with reverse primers IM650R
231 and TY282Rd, 54°C with primer TY282Rnd and 55°C with primer TY153Rnd - and 45 s elongation at 72
232 °C. The PCR program was ended by a 5- or 10-min incubation at 72 °C. The PCR products were resolved

233 by electrophoresis in a 1% to 2% agarose gel according to the different length of the fragment, and
234 visualized with ethidium bromide staining.

235 The Srec/Lrec discriminating PCR test was validated with total DNA extracted from tomato plants
236 agroinoculated with infectious viral clones (Fig. 1). Thus, the Srec PCR test was validated from total
237 DNA of a tomato plant of the cultivar Pristyla (Gautier Semences, France) agroinfected with TYLCV-
238 IS76 (Belabess et al., 2015), and total DNA from a tomato plant of the cultivar Calvi (Gautier Semences)
239 agroinfected with TYLCV-IS141 (TYLCV-IS141[IT:Sic1; 13]) (Urbino et al., 2020). The Lrec-IL PCR test was
240 validated with a total DNA from a tomato plant of the cultivar Pristyla co-agroinoculated with TYLCV
241 and TYLCSV (Belabess et al., 2018); the DNA extract was from plant R11 in which Lrec recombinants
242 were detected by Sanger sequencing in a sample collected 180 days post inoculation [see Fig. 8 of
243 (Belabess et al., 2018)]. The Lrec-Mld PCR test was validated with a recombinant plasmid DNA
244 containing the full-length genome of a TYLCVMalV clone (Genbank accession number LN846611)
245 (Belabess et al., 2015); the viral clone was obtained from a bean plant (H5) sampled in March 2012 in
246 the Souss region of Morocco. Total DNA extracted from a tomato plant of the cultivar Pristyla
247 infiltrated with an agrobacterium clone carrying an empty PC2300 binary vector was used as negative
248 control.

249 **Figure 1**: **Presentation of PCR primers designed to distinguish Srec and Lrec recombinants**. (A) PCR
250 primers targeting recombinants that inherited short TYLCSV derived fragments of less than 623 nts
251 (Srec). (B) PCR products obtained with Srec-targeting primers resolved on a 1.5% agarose and
252 stained with ethidium bromide (M, Benchtop ladder, Promega, France). (C) PCR primers targeting
253 recombinants that inherited TYLCSV-derived fragments of more than 623nts (Lrec). The reverse
254 primer IL-2258R, specifically hybridize to Lrec having a TYLCV-IL parental virus, whereas Mld-2310R
255 specifically hybridize to Lrec having a TYLCV-Mld parent. (D) PCR products obtained with Lrec-
256 targeting primers resolved on a 1% agarose gel (M, Gene ruler 1kb, Fisher Scientific SAS, France).
257 All PCR primers are described in Table 1. IS76, TYLCV-IS76; IS141, TYLCV-IS141; X, non-used wells;
258 PC, total DNA from a mock inoculated plant.

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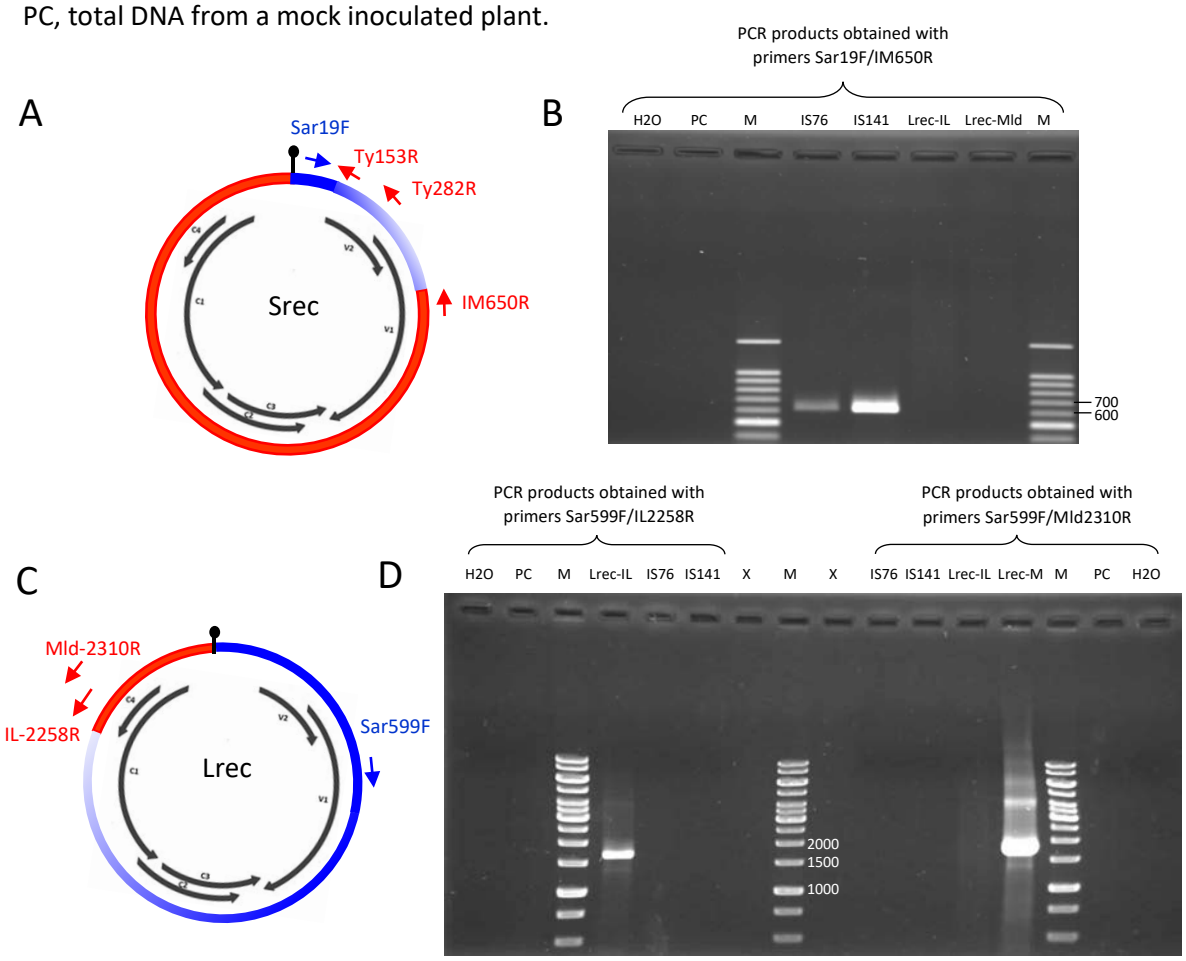

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274 **Table 1**: **Description of PCR primers and primer products.**

Name of PCR test Primer Name	Sequence	Targeted region (Genomic coordinates)[2]	Recombinant type detected	Size of expected product
Srec specific PCR				
Sar19Fd[1]	5'-CCGATAAAGWAGTRGGHCCTACGCA-3'	IR (19-43)	Forward primer to detect SRec	-
Sar19Fnd[1]	5'-CCGATAAAGTAGTGGGCCCTACGCA-3'	IR (19-43)		
Ty282Rd[1]	5'-CACGKGCYCTTACAACRGATATA-3'	V2 (296-274)	Reverse primer to detect SRec with TYLCSV-derived fragments of less than 274 nucleotides like IS76 and IS141	278bp
Ty282Rnd[1]	5'- CACGGGCCCTTACAACAGATATA -3'	V2 (296-274)		
TY-153Rnd[1]	5'-AGTGGGTCCCACATATTGCAAGAC-3'	IR/V2 (153-130)	Reverse primer to detect SRec with TYLCSV-derived fragments of less than 130 nucleotides like IS76	135bp
IM-650R	5'-TCGATTTAACACAGAACCTC -3'	V1 (642-623)	Reverse primer to detect SRec with TYLCSV-derived fragments of less than 530 nucleotides	624bp
Lrec specific PCR				
Sar599F	5'- GGTATTACWCATAGAGTWGGTAAAC-3'	V1 (599-623)	Forward primer to detect LRec-IL and LRec-Mld	-
IL-2258R	5'-TCARCTAGAGGAGGTCARCAATCT-3'	C1 (2250-2227)	Reverse primer to detect LRec-IL	1652bp
Mld-2310R	5'-CGATAAGGACGGARACACCG-3'	C1 (2310-2291)	Reverse primer to detect LRec-Mld	1712bp

275 [1] Primers reported in Belabess et al. (2018). [2] Nucleotide coordinates of the primers are referred to the genome of TYLCV-IS76 [MA:SouG8:10] (GenBank
276 accession number LN812978), except for primer Sar599F for which they refer to the genome of TYLCSV-ES [MA:Aga5a:12] (GenBank accession number
277 LN846598) and primer Mld-2310R to the genome of TYLCV-Mld[RE:02] (GenBank accession number AJ865337). Position 1 is always the position of the
278 first nucleotide to be replicated at the origin of replication.
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282 Amplification products generated with Srec/Lrec discriminating PCR primers are presented in Fig. 1B
283 and Fig. 1D. Srec primers do not generate any amplification product with Lrec infected plants and vice
284 versa. No amplification product was detected with a mock-inoculated plant infiltrated with the
285 agrobacterium clone carrying an empty PC2300 binary vector.

286 The Srec/Lrec discriminating PCR test was subsequently used to analyse tomato samples detected
287 positive for the presence of TYLCV/TYLCSV recombinants according to the results obtained with the
288 MxPCR1 test (Belabess et al., 2015) targeting recombination breakpoints at the origin of replication.

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290 3.2. Infection profiles of samples collected in Morocco

291 Among the 51 samples analysed, 46 were detected positive for at least one PCR-targeted virus and
292 three infection profiles were distinguished (Table 2): TYLCSV (1 sample), IL/Sar (43 samples), and
293 coinfection with IL/Sar and Mld/Sar (2 samples). Moroccan samples can be characterized as follow. (i)
294 The parental virus types TYLCV-IL and TYLCV-Mld were not detected and the TYLCSV type was detected
295 in only one sample. (ii) All the 46 PCR-positive samples, were positive for IL/Sar except the sample
296 detected positive for TYLCSV only. (iii) The recombinant-positive samples were negative for
297 recombinant-types that inherited long TYLCSV fragments (Lrec-IL), but positive for Srec recombinants.
298 (iv) The recombination breakpoints within the sequence of the Srec PCR products were analysed with
299 5 samples of different regions and collection years, and were shown to be all at position 76 like that of
300 the typical recombination breakpoint of TYLCV-IS76.

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303 **Table 2: Begomovirus infection profiles of tomato plants sampled in Morocco, Italy and Spain.**
304 Total DNA of plant samples were analysed by PCR and DNA sequencing. The presence of Tomato
305 yellow leaf curl virus (TYLCV), Tomato yellow leaf curl Sardinia virus (TYLCSV) and the interspecies
306 recombinants derived from them were tested with the multiplex PCR MxPCR1 and MxPCR2
307 (Belabess et al., 2015); note that the test distinguishes the Israel and Mild strain of TYLCV and the
308 recombinants derived from them, TYLCV-IL/TYLCSV (IL/Sar) and TYLCV-Mld/TYLCSV (Mld/Sar).
309 "Sar/Ty" corresponds to potential reciprocal recombinants TYLCSV/TYLCV. Samples from Spain
310 detected positive for TYLCV-IL were analysed further by sequencing to specify to which formerly
311 identified group they may belong to (Torre et al., 2018), i.e., group 1 (g1) or group 2 (g2) as shown
312 in Fig.3. Samples detected positive for recombinants were analysed further by PCR tests to
313 distinguish them according to the size of the fragment inherited from TYLCSV, either long (Lrec-IL
314 or Lrec-Mld) or short (Srec) (Fig. 1, Table 2). Positions of recombination breakpoints were identified
315 by sequencing PCR products (last 3 columns); the number of "+" indicates the number of samples
316 that were analysed by sequencing. Recombination breakpoints (RB) were detected at position 60,
317 76, or 141. [1]Samples for which recombinant genomes were totally sequenced following cloning; the
318 three sequences of the Sardinian TYLCV-IS141 were reported previously (Granier et al., 2019). In
319 the columns corresponding to PCR tests, + and - indicate that a PCR product of the expected size
320 was detected or not detected, respectively; an empty box means that the PCR test was not done.
321 The boxes containing a "+" were coloured in yellow to facilitate the reading of the table. In the
322 column describing the samples, NA means not available.

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Origin	Sampling date	Region	Tomato genotype or cultivar (TYLCD resistance status)	TYLCD symptoms	Number of samples/ infection profile	MxPCR2 (Belabess et al. 2015)			MxPCR1 (Belabess et al. 2015)			PCR tests (Fig.1)			Partial genome sequence		
						TYLCV -IL	TYLCV -Mld	Sar/Ty	TYLCSV	IL/Sar	Mld/Sar	Lrec -IL	Lrec - Mld	Srec	RB60	RB76	RB141
Morocco (Atlantic coastal region)	Dec 2015	Agadir	Breeding material (NA)	NA	1	-	-	-	+	-	-						
					1	-	-	-	-	+	-	-		+		+	
					1	-	-	-	-	-	-						
	Jan-Apr 2017	Oualidia	Antalya (S)	+	6	-	-	-	-	+	-	-		+			
					2	-	-	-	-	+	+	-	-	+			
			Calvi (S)		8	-	-	-	-	+	-	-		+		+	
		Safi doukkala had Ihrara	Calvi (S)		4	-	-	-	-	+	-	-		+		+	
		Chtouka ouled laissaoui	Calvi (S)		4	-	-	-	-	+	-	-		+			
		Ouled Frej	Calvi (S)		8	-	-	-	-	+	-	-		+			
			Pristyla (Ty-1)		4	-	-	-	-	-	-						
		Azmour	Dynamite (S)		4	-	-	-	-	+	-	-		+		+	
		Agadir	Calvi (S)		4	-	-	-	-	+	-	-		+		+	
		Agadir	Wafira (S)		4	-	-	-	-	+	-	-		+			
Italy (Sardinia)	Sept 2016	NA	Parioli (Ty-1)	-	1	-	-	-	+	-	-						
				+	2	-	-	-	+	+	-	-		+			++[1]
					1	+	-	-	+	+	-	-		+			+[1]
Italy (Sicily)	Nov 2015	NA	Breeding material (NA)	NA	2	-	-	-	-	+	+	-	-	+			++
	Dec 2016	Gela	Pakita (NA)	yellowing	1	+	-	-	-	+	+	-	-	+			
		Marina di Ragusa	Ikram (S)	+	1	-	-	-	-	+	-	-		+	+		
		Gela	Creativo (Ty-1)	yellowing	1	+	-	-	-	+	-	-		+			+

	Dec 2017	Pachino	Breeding material (NA)	NA	6	-	-	-	-	-	-						
					1	+	+	-	-	+	+	-	-	+			+
					1	-	-	-	-	-	+		-	+			+
			Creativo, C113 (Ty-1)		2	-	-	-	-	+	-	-	-	+			++
	Oct 2018	NA	NA (NA)	NA	3	-	-	-	-	+	-	-	-	+	+++[1]		
					2	-	-	-	-	+	-	-	-	+			++
	Oct 2019	Vittoria	Breeding material (NA)	NA	9	+	-	-	-	+	+	-	-	+			++
					2	-	-	-	-	+	-	-	-	+	+		
					2	-	-	-	-	+	+	-	-	+			
Spain (Southeast)	Dec 2015	Almeria	6304 La Canada (NA)	NA	1	+ (g1)	-	-	-	-	-						
			Breeding material (NA)		1	+ (g1)	-	-	-	-	-						
	Feb 2016	Almeria (El Ejido)	Ateneo (Ty-1)	+	2	+ (g1)	-	-	-	-	-						
			Caniles (Ty-1)	+	1	+ (g1)	-	-	-	-	-						
	Sep 2017	Almeria	Breeding material (NA)	+	1	+ (g1,g2)	-	-	-	-	-						
					2	+ (g2)											
	Oct 2018	Almeria (El Ejido)	7966 (NA)	?	1	-	-	-	-	+	-	-		+		+	
		Murcia	L4238.0 (Ty-2?), C4239.0 (Ty-1)	+	2	-	-	-	-	-	-						
		Murcia	Calvi (S), Pristyla (Ty-1)	+	2	+ (g1)											
		Murcia	Myriade (S); Faustyno, C113 (Ty-1)	+	3	+ (g2)	-	-	-	-	-	-					
		Murcia	Vinety (Ty-1)	+	1	-	-	-	-	+	-	-		+		+	

329 The 4 samples collected in Ouled-Frej from symptomatic Ty-1 resistant tomato plants of the cultivar
330 Pristyla tested negative for all targeted begomoviruses whereas all the 8 samples from the susceptible
331 cultivar Calvi were detected positive. It is possible that the PCR tests were not sensitive enough to
332 detect the low virus amounts of Ty1 resistant Pristyla plants which were reported to be about 10 times
333 lower than the amounts in nearly isogenic susceptible tomato plants (Belabess et al., 2016). This
334 hypothesis is consistent with the observation of a relatively faint band observed in lane IS76 of the
335 agarose gel shown in Fig. 1A, corresponding to an amplification product obtained with the Srec specific
336 primers, from a Pristyla tomato plant agroinoculated with a clone of TYLCV-IS76. It is also possible that
337 the milder TYLCD symptoms exhibited by Ty-1 resistant plants may have been confused with other
338 types of symptoms.

339

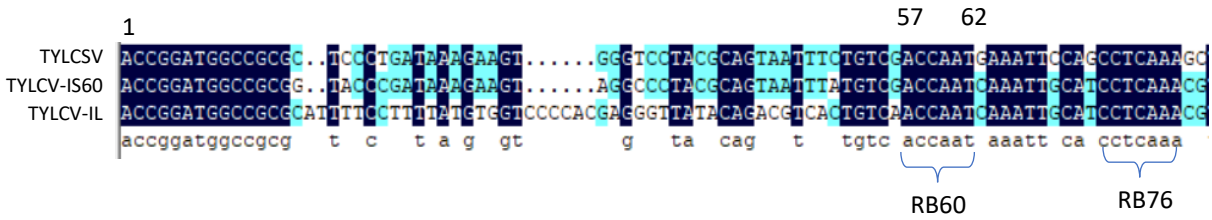
340 3.3. Infection profiles of samples collected in Italy

341 Among the 37 samples analysed, 31 were detected positive for at least one PCR-targeted virus. The
342 infection profiles are relatively heterogeneous. Indeed, unlike in Morocco where only one parental-
343 type virus was detected (TYLCSV) and only one type of TYLCV/TYLCSV recombinant (TYLCV-IS76), in
344 Italy, all three parental type viruses were detected as well as two types of recombinants, TYLCV-IS141
345 and a new one described below, TYLCV-IS60 (IS60). Moreover, whereas only three infection profiles
346 were detected in Moroccan samples, up to 9 infection profiles were detected in Italian samples. In
347 spite of these differences between infection profiles of Italian and Moroccan samples, some common
348 features can be noted. Thus, like in Morocco, only one sample was infected with a parental virus alone
349 (TYLCSV), whereas all the other samples detected PCR-positive for at least one of the targeted
350 begomovirus-types, were positive for the presence of IL/Sar or Mld/Sar recombinants. Moreover, like
351 in Morocco, all the recombinant-positive samples are negative for Lrec recombinants, but positive for
352 Srec recombinants.

353 To further characterize the Srec recombinants, 19 of the 30 Srec-positive samples were further
354 analysed by sequencing; samples were selected from each collection year and from different sampling
355 locations. The sequencing results revealed that 14 samples contained Srec recombinants of the IS141-
356 type with a recombination breakpoint around position 141, whereas 5 samples contained a new type
357 of Srec recombinants with a recombination breakpoint located within the 57 to 62 position-range of
358 nucleotides that are identical between TYLCV and TYLCSV parental genomes (Fig. 2). Hence it was
359 named TYLCV-IS60 according to the coding used to name previous Srec-type recombinants (Belabess
360 et al., 2015), 60 being one of the two median positions of the 6 non-discriminating nucleotide range in
361 which recombination events occurred. IS60 recombinant genomes were cloned from three samples
362 collected in October 2018 and were fully sequenced (GenBank accession numbers OR473055,
363 OR473056, OR473057). Besides the recombination breakpoint at the origin of replication and around
364 position 60, no other recombination breakpoint was detected. The IS60 genomes are 2783 or 2784 nts
365 in length and differ from each other at 1, 8 or 9 positions. Whereas IS141-type recombinants were
366 detected every year since 2015, IS60-type recombinants were detected only in samples collected in
367 2016, 2018 and 2019.

368 Six samples collected in Pachino on symptomatic plants from breeding material tested negative. As
369 suggested with symptomatic samples from Ty-1 resistant plants collected in Ouled-Frej (Morocco), the
370 6 Pachino samples may have been collected from resistant plants in which the virus amount was too
371 low to be detected. It is also possible that the supposed TYLCD symptoms were caused by some other
372 stresses.

Figure 2: **DNA alignments showing the typical recombination breakpoint of the TYLCV-IS60 recombinant.** Partial DNA sequences of TYLCSV-Sic (GenBank accession number GU951759), TYLCV-IL from Italy (GenBank accession number DQ144621) and one representative of the TYLCV-IS60 (GenBank accession number OR473055) were aligned. Number 1 is the nucleotide position of the origin of replication, and positions 57 to 62 is the range of non-discriminating nucleotides bearing the recombination breakpoint RB60 typical of TYLCV-IS60. The range of non-discriminating positions bearing RB76 is shown for information. The numbering is according to the TYLCSV sequence.



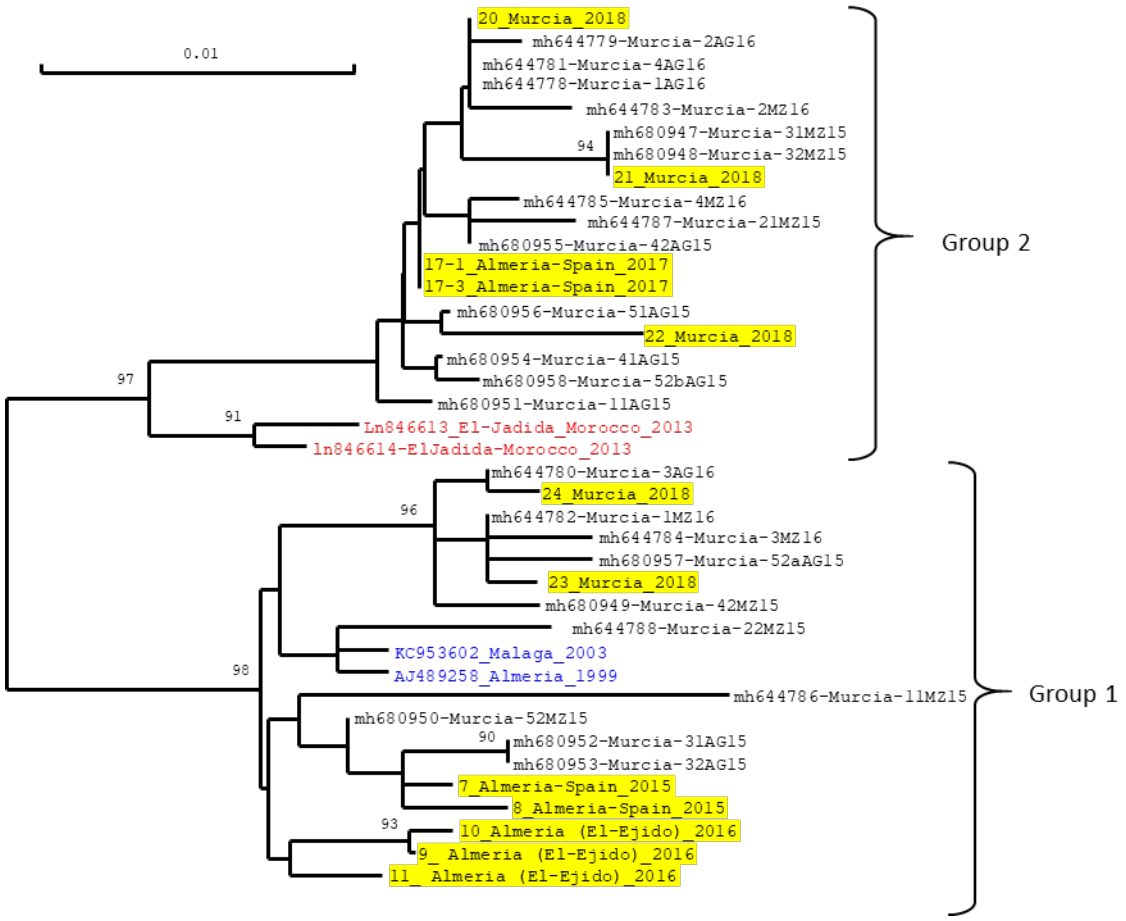
3.4. Infection profiles of samples collected in Spain

Among the 17 samples analysed, 15 were detected virus- positive and all of these 15 plants exhibited a mono-infection profile. Thus, thirteen samples were positive only for the presence of TYLCV-IL, and two only for TYLCV-IS76-type recombinants. Whereas TYLCV-IL was detected every year of sampling between 2015 and 2018, TYLCV-IS76-type recombinants were detected in 2018 only.

The TYLCV-IL-positive samples were analysed further by sequencing a PCR product containing nucleotide positions that can be used to discriminate two TYLCV strains previously reported from Southeast Spain as group 1 and 2 (Torre et al., 2018). Viruses infecting the 5 TYLCV-IL positive plants sampled in 2015 and 2016 clustered with group 1 viruses (Fig. 3); two TYLCV-IL-positive samples collected later (2018) were found to be infected with group 1 viruses too. Viruses infecting all the other TYLCV-IL-positive samples, i.e., 2 collected in 2017 and 3 collected in 2018, clustered with group 2 viruses. Interestingly, one of the three samples collected in 2017 was found to be co-infected with group 1 and group 2 viruses. Indeed, the Sanger-sequencing electropherogram obtained with this sample exhibited double peaks at group1/group2 discriminating positions.

Like the 4 samples collected in Ouled-Frej (Morocco) that tested negative in spite of their collection on symptomatic plants, 2 samples collected in Murcia in Oct 2018 revealed the same inconsistency (Table 1). As suggested for the Ouled-Frej samples, the PCR tests were not sensitive enough to detect the potentially lower virus amount that is expected in resistant plants. It is also possible that the milder TYLCD symptoms exhibited in Ty-1 resistant plants may have been confused with other types of symptoms.

Figure 3: **Neighbour-joining tree showing the genetic distance among isolates of Tomato yellow leaf curl virus (TYLCV) originating from Southeast Spain**. The tree was constructed from the alignment of a 592-nt-genomic fragment between the origin of replication and the first third of the capsid protein gene. The sequences used for the comparison were from viruses isolated in this study (highlighted in yellow) as well as from other isolates for which sequences were downloaded from Genbank. All the isolates were from tomato plants (*Solanum lycopersicum*) except the isolates collected in 1999 and 2003 which were collected from pepper (*Capsicum annuum*) (written in blue). Sequence data from this study were obtained by sequencing a PCR product generated with the primer pair IL-2629F/ IM650R, and the sequences of the 592-nt fragment are available in Supplementary material 1. Virus isolates from this study are identified with a code number followed by the location and year of collection. Isolates written in black without yellow highlights were collected in the province of Murcia and are identified with their GenBank accession number and the code numbers used previously, in which 15 and 16 correspond to the year of collection (2015, 2016) and AG and MZ to the sampling locations, i.e., Aguilas and Mazaron, respectively (Torre et al., 2018). Isolates written in blue correspond to the earliest collected TYLCV in Southeast Spain for which total genome sequences were reported. Isolates written in red correspond to the two TYLCV isolates from El-Jadida (Morocco) that were reported to be the most closely related to group 2 isolates identified as a new type of TYLCV isolates in Southeast Spain (Torre et al., 2018). The scale measures the Jukes and Cantor distance between sequences. Numbers associated with nodes represent the percentage of 1000 bootstrap iterations supporting the nodes; only percentages ≥ 90% are represented. The two highly supported groups correspond to the previously identified group 1 and group 2 (Torre et al., 2018).



453 **4. Discussion**

454 Begomovirus populations associated with TYLCD in western Mediterranean countries are composed
455 of viruses belonging to the alien species *Tomato yellow leaf curl virus*, the native species *Tomato yellow*
456 *leaf curl Sardinia virus*, and various interspecies recombinants. Although representatives of each of
457 these three TYLCD-associated virus types were reported from Spain, Italy and Morocco, country
458 specificities were previously detected with plant samples collected over time but mostly before 2015;
459 sampling efforts extended up to 2003 in Spain (Garcia-Andres et al., 2007), up to 2008 in Italy (Davino
460 et al., 2009) and up to 2014 in Morocco (Belabess et al., 2015). Considering geographic proximity and
461 trade activities between these countries, as well as the efficient whitefly transmission of these viruses,
462 it was not known if these specificities would be maintained. This question was addressed here by
463 analysing the infection profile of samples collected between 2015 and 2019 in the three countries.

464 Due to their resistance breaking feature in Ty1 resistant plants (Belabess et al., 2015; Fortes et al.,
465 2023; Panno et al., 2018), Srec recombinants were given special attention by distinguishing them with
466 an original PCR test from Lrec recombinants commonly reported from Spain and Italy (Davino et al.,
467 2012; Garcia-Andres et al., 2006; Monci et al., 2002). Thus, together with the previously reported
468 multiplex PCR tests (Belabess et al., 2015), we were able to specifically determine the proportion of
469 samples containing Srec recombinants among the samples detected positive for TYLCD-associated
470 begomoviruses.

471 4.1. TYLCV-IS76 is virtually the only TYLCD-associated begomovirus on the Atlantic coast of Morocco

472 The dominance of Srec recombinants in the samples collected from Morocco and their identification
473 as TYLCV-IS76 is fully consistent with previous results (Belabess et al., 2015). Indeed, these authors
474 showed that TYLCV-IS76 has replaced its parental viruses in the region of Agadir (Souss) and that it
475 started to spread northwards. The northward spread is confirmed here with Srec recombinants being
476 the only begomovirus type detected in the 10 farms located in the north of Agadir along the Atlantic
477 coast up to Casablanca. The detection of TYLCSV in 2015 in Agadir is consistent with previous results
478 showing that TYLCSV was more frequently detected than TYLCV among the rare tomato samples in
479 which parental type viruses were detected after the invasion of TYLCV-IS76 (Belabess et al., 2015).
480 Further studies are needed with plant samples from northern and eastern Morocco to complete the
481 picture of tomato begomoviruses in Morocco.

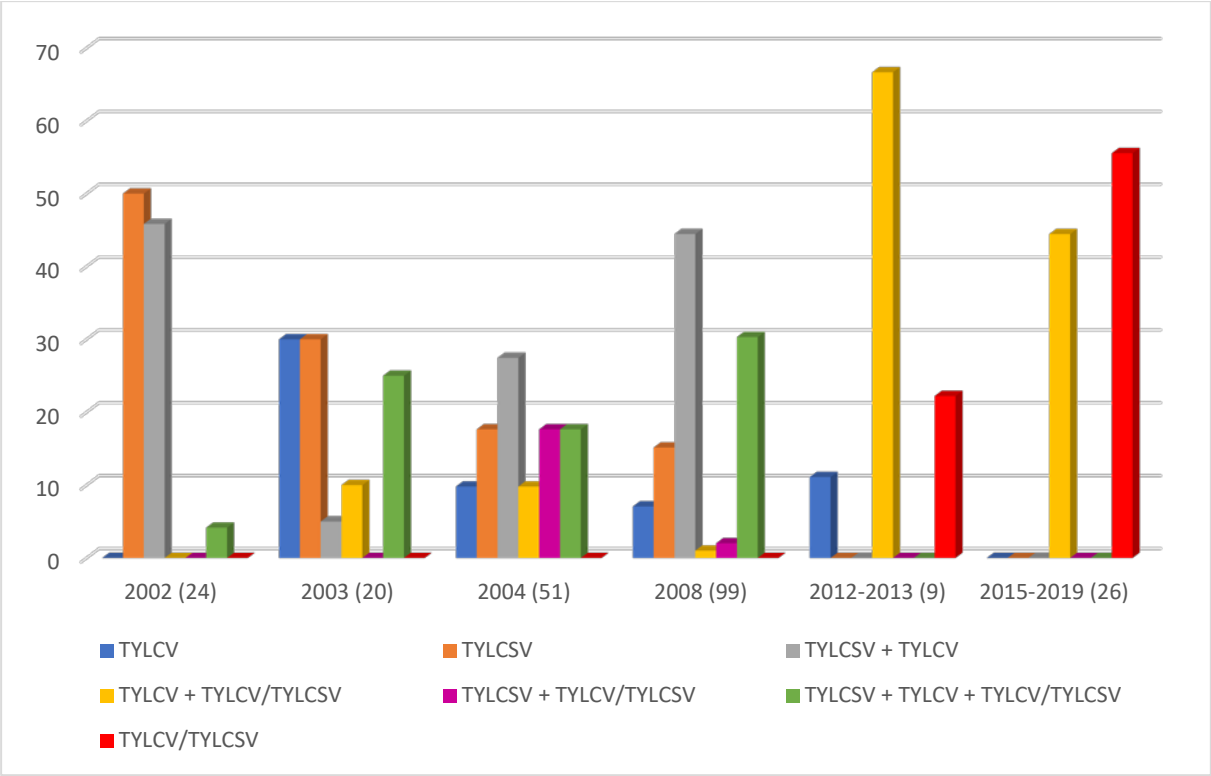
482 4.2. Srec recombinants are present in virtually all begomovirus-positive samples in Italy

483 Most of the samples collected from Italy are from Sicily. One of the major results with Sicilian samples
484 is that all the plants samples were positive for TYLCV/TYLCSV recombinants, comprising 11 samples in
485 which the TYLCV parent of the recombinants is of the IL strain, 1 sample in which it is of the Mld strain,
486 and 15 samples in which both type of recombinants were detected, i.e., IL/Sar and Mld/Sar. These
487 infection profiles can be compared with those determined from tomato samples collected in Sicily
488 before 2015 (Belabess et al., 2015; Davino et al., 2008; Davino et al., 2009) because similar PCR
489 approaches were used (Fig. 4). However, as the PCR techniques used by Davino et al were not designed
490 to distinguish IL and Mld strains (Davino et al., 2008; Davino et al., 2009), the comparison was done
491 after grouping IL and Mld strain-positive samples - distinguished in our study and in that of Belabess
492 et al. - in a unique TYLCV group, and similarly, after grouping IL/Sar and Mld/Sar recombinants in a
493 unique TYLCV/TYLCSV group. In 2002, shortly after the invasion of TYLCV, only three types of infection
494 profiles were detected. Subsequently, the number of infection profiles increased with a maximum of
495 6 infection profiles in 2004 and 2008. Such highly diverse infection profiles were confirmed from
496 samples collected in Sicily between 2006 and 2009, at the rate of 100 samples per year (Davino et al.,
497 2012). In spite of high sampling efforts, samples that were positive only for TYLCV/TYLCSV

recombinants ("TYLCV/TYLCSV") were not detected before 2009. An important change has apparently occurred between 2009 and 2012, because among the 9 samples collected in the 2012-2013 period, 2 samples exhibited the "TYLCV/TYLCSV" infection profile (Belabess et al., 2015). In samples collected later, between 2015 and 2019, that were analysed in this study, "TYLCV/TYLCSV" was the dominant infection profile, suggesting that recombinants with a relatively higher fitness have emerged. Noteworthy, while the recombinants reported from Sicily before 2012 were all of the Lrec type (Davino et al., 2012; Davino et al., 2009), the recombinants detected in the samples collected during 2015-2019, were all of the Srec type (Table 1) and 5 out of the 9 samples collected in 2012-2013, were positive for Srec too (Belabess et al., 2015). All together these results suggest that the relatively higher fitness of the recombinants detected since 2012 is determined by their typical recombination profile. This conclusion is supported by the identification of TYLCV-IS141 in most of the samples that were analysed further by sequencing [Table 1 and (Belabess et al., 2015)], a recombinant whose fitness was reported to be similar to that of TYLCV-IS76, the invasive recombinant that emerged in Morocco (Urbino et al., 2020).

Unlike Sicilian samples which were all negative for TYLCSV, the four Sardinian samples were all positive for TYLCSV. However, like in Sicily, all the samples detected positive of TYLCV/TYLCSV recombinants were positive for Srec but negative for Lrec. Although further conclusions cannot be drawn from such a limited number of samples, these results show at least that the prevalence of TYLCD associated begomoviruses in Sardinia cannot be extrapolated from the results obtained from Sicily where TYLCSV was not detected in any of the 33 samples analysed.

Figure 4: **Dynamics of the relative frequency of plants exhibiting particular profiles of begomovirus infections in Sicily.** The infection profile data of the samples collected between 2015 and 2019 were extracted from Table 1, whereas those of samples collected earlier were extracted from previous reports (Belabess et al., 2015; Davino et al., 2008; Davino et al., 2009). The data used to generate Fig. 4 are available in Supplementary material 2. As similar PCR approaches were used in previous and present studies and as all the begomovirus types targeted in the previous studies were also targeted here, the results are presented on the same graph. The figure presents the percentage of plants exhibiting each infection profile over time. The number of plants analysed in each collection period are indicated in brackets under the graph. The PCR tool used by Davino et al. was not specific enough to distinguish the two strains of TYLCV (IL and Mld) and their recombinants with TYLCSV. Hence, for the sake of homogeneity and clarity, the figure does not distinguish the IL and Mld strains for the samples analysed in this study (Table 1) and by (Belabess et al., 2015); IL and Mld strains were combined in a unique TYLCV group and similarly the recombinants IL/Sar and Mld/Sar were combined in a unique TYLCV/TYLCSV recombinant group. Noteworthy, it is only from the sampling of 2012-2013 that some samples were detected positive only for TYLCV/TYLCSV recombinants.



4.3. TYLCV-IS60, a new Srec recombinant spreading in Sicily

Interestingly, besides TYLCV-IS141, a second Srec recombinant was detected in Sicily during the period 2015-2019, i.e., TYLCV-IS60. Although TYLCV-IS60 was detected less frequently than TYLCV-IS141 (Table 1), it exhibits features of an emerging virus. Indeed, the 4 samples that were positive for TYLCV-IS60 were not positive for any of the targeted begomoviruses, indicating that TYLCV-IS60 is already circulating autonomously. Moreover TYLCV-IS60 was detected from samples collected in three of the five years of sampling, and it was detected in at least two distinct collection sites, namely Marina di Ragusa and Vittoria.

4.4. Emergence of TYLCV-IS76 and its presumed back recombinant in Southeast Spain

The dominant infection profile of the samples collected in the Murcia and Almeria provinces is “TYLCV” with 13 samples being positive for TYLCV only, among the 15 PCR-positive samples (Table 1). This result is consistent with previous reports with samples collected earlier from the same Spanish region. Thus, the five tomato samples collected in 2013 in Almeria province and analysed with the same PCR approach were PCR positive only for TYLCV (Belabess et al., 2015). Likewise, among 370 tomato samples collected in the two Spanish provinces in 2003 and detected positive for tomato begomoviruses by tissue blot hybridization, 368 were positive only with the DNA probe specific to TYLCV; 2 were positive with both the TYLCV and the TYLCSV probes and no sample was detected positive for TYLCSV only (Garcia-Andres et al., 2007). In this later study in which tomato samples were also analysed from the Malaga province, 45 tomato samples from the three provinces, detected positive by tissue-blot hybridization, were selected to be analysed further by sequencing a PCR product comprising the non-coding intergenic region and the 5’-proximal parts of Rep and V2 open reading frames. Among them, only three samples bore TYLCV/TYLCSV recombinant genomes, two which inherited the TYLCV fragment from a Mld strain parent and one from an IL strain parent. This low proportion of recombinants stands in contrast with the 2 samples detected positive for IL/Sar

562 recombinants among the 15 PCR positive samples analysed here. The much higher prevalence of
563 TYLCV/TYLCSV recombinants in tomato is apparently related with the length of the TYLCSV fragment
564 inherited by the recombinants. Whereas the 2 recombinants detected in 2018 have inherited a short
565 76 nts fragment of TYLCSV, like that of the previously reported TYLCV-IS76 viruses, the three
566 recombinants detected in 2003 inherited a much longer TYLCSV fragment that extended beyond the
567 initiation codon of the V2 gene. While the TYLCV-IS76-positive sample form El Ejido confirms the
568 emergence of TYLCV-IS76 in the Almeria province (Fortes et al., 2023; Torre et al., 2019), the detection
569 of TYLCV-IS76 in a sample from Murcia shows that its distribution is not limited to the Almeria province
570 (Table 1).

571 The high nucleotide identity between TYLCV-IS76 recombinants from Spain and Morocco (Fortes et al.,
572 2023; Torre et al., 2019) suggests that their presence in Spain is due to an introduction from Morocco
573 where it was initially detected in tomato plants sampled in 2010. Such a scenario may also explain the
574 presence of a new type of TYLCV-IL viruses in Southeast Spain because of their close nucleotide identity
575 to TYLCV-IL viruses detected in Morocco from tomato plants sampled in 2013 in El Jadida (Fig. 3). Based
576 on a high sequence identity with the TYLCV-inherited region of TYLCV-IS76, El Jadida-TYLCV-IL-like
577 viruses were presumed to derive from a TYLCV-IS76 recombinant through a back recombination event
578 (Belabess et al., 2015; Torre et al., 2018). Their detection in more than 50% of the TYLCV-IL-positive
579 samples collected in 2017 and 2018, confirm their emergence in Southeast Spain since their first
580 detection from tomato samples collected in 2015 and 2016 (Torre et al., 2018). As these authors have
581 analysed only samples from the province of Murcia, it was not known if the El Jadida-TYLCV-IL-like
582 viruses have emerged elsewhere. Here, with samples collected in 2017 in the province of Almeria
583 (Table 1), we show that their presence is not limited to the province of Murcia. The successful
584 emergence of viruses of the El Jadida-TYLCV-IL-type is consistent with their fitness advantage shown
585 with a Spanish clone inoculated in Ty-1 resistant tomato plants (Torre et al., 2018). Indeed, the El-
586 Jadida type clone was significantly more accumulated than TYLCV-IL and TYLCV-IS76 in mono-infected
587 plants. Considering the emergence of the El Jadida-TYLCV-IL-like viruses in Spain, it is puzzling that they
588 were not detected in any of the plants that were sampled in 2015 and 2017 in the region of El Jadida;
589 indeed, all the Moroccan samples tested negative for TYLCV-IL (Table 1). It is possible that their
590 emergence was impeded in Morocco due to the dominance of TYLCV-IS76 which was shown to be
591 more competitive than the El Jadida-TYLCV-IL when representatives of both types were co-inoculated
592 in tomato plants, irrespective of the cultivar, with or without the Ty-1-resistance gene (Torre et al.,
593 2018).

594 4.5 Concluding remarks

595 The populations of TYLCD-associated begomoviruses that are described in this study reveal that
596 country specificities were still present between 2015 and 2019. The specificities concerning parental
597 type viruses (TYLCV, TYLCSV) is their virtual absence in the sampled region of Morocco along the
598 Atlantic coast, the relatively frequent detection of both parents in Italy but with potential regional
599 specificities (Sicily/Sardinia), and frequent detection of TYLCV in Spain. The specificities concerning
600 recombinants is revealed by the dominance of TYLCV-IS76 in Morocco but with no detection of TYLCV-
601 IS141, the dominance of TYLCV-IS141 in Italy but with no detection of TYLCV-IS76, and confirmation of
602 the emergence of TYLCV-IS76 and the El Jadida-TYLCV-IL-type in Southeast Spain.

603 However, besides these country specificities, a general trend was revealed with the original PCR test
604 developed here to distinguish Srec and Lrec recombinants. Indeed, while TYLCV/TYLCSV recombinants
605 detected in tomato samples collected before 2010 were all of the Lrec type (Belabess et al., 2015;
606 Davino et al., 2008; Davino et al., 2012; Davino et al., 2009; Garcia-Andres et al., 2007; Garcia-Andres
607 et al., 2006; Monci et al., 2002), all the tomato samples detected positive for TYLCV/TYLCSV

recombinants in this study are positive for Srec type recombinants and negative for Lrec. Thus, since the first detection of Srec type from Morocco in 2010 and from Sicily in 2012 (Belabess et al., 2015), the prevalence of Srec recombinants has increased at the expenses of Lrec recombinants.

The higher competitiveness of TYLCV-IS76 as compared to Lrec recombinants observed in controlled conditions using vector transmission (Jammes et al., 2023) is consistent with these inverse dynamics of prevalence of Srec versus Lrec recombinants. The large regional scale at which this trend is detected may be explained by the cultivar switch that occurred in the three countries from susceptible to Ty-1 resistant cultivars, as reported from Morocco (Belabess et al., 2015; Belabess et al., 2016). More recent samplings are needed to monitor further the evolution of TYLCD-associated begomoviruses in these countries and particularly the dynamics of TYLCV-IS76 and El Jadida-TYLCV-IL-type in Spain, the fate of the new Srec recombinant circulating in Sicily (TYLCV-IS60), and the potential persistence of parental type viruses in the less intensive tomato production areas such as the North East of Morocco.

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Conflict of interest disclosure

The authors declare that they have no financial conflicts of interest in relation to the content of the article

Author contributions

Mohamed Faize (MF) organized the collection of Moroccan samples. Martine Granier (MG) and Sandie Passera extracted total DNA from plant samples. MG performed the laboratory analysis of the samples. Michel Peterschmitt (MP) designed the study, analysed the data and wrote the original draft. MF, Cica Urbino and MP reviewed and edited the manuscript.

Data

DNA sequences of the three complete genomes, generated in this study for the newly reported TYLCV-IS60 recombinant, are available in GenBank under accession numbers OR473055, OR473056, OR473057. The partial sequences of other viruses generated in this study and used to construct the distance tree shown in Fig. 3 are available in the Supplementary material 1. The data used to prepare Fig. 4 are available in Supplementary material 2.

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Supplementary material 1: DNA sequences of the 592-nt-fragment used to compare the 13 TYLCV isolates detected in tomato samples collected in Southeast Spain (Table 1) with samples collected in other studies and particularly in (Torre et al., 2018). Only 12 of the 13 sequences generated from TYLCV isolates from Spain are presented, because one of the three samples collected in the province of Almeria in 2017 (Table 1) was co-infected with group 1 and group 2 viruses; indeed, the electropherogram revealed double peaks at nucleotide positions at which the two types of viruses can be distinguished.

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884 **Supplementary material 2**: The data presented in this table were used to generate Fig. 4. They
885 present the number of tomato plants of each infection profiles sampled in different years in Sicily.
886 The data obtained with plants sampled in the years 2002 and 2004 are from [(Davino et al., 2008),
887 Table 2], those from years 2004 and 2008 are from[(Davino et al., 2009), Table 2], those from years
888 2012- 2013 are from [(Belabess et al., 2015), Supplementary Table S8], and those from years 2015-
889 2019 are from this study (Table 1). Table cells corresponding to plants detected positive for
890 TYLCV/TYLCSV recombinants are highlighted in orange. The penultimate table line shows the total
891 number of plants detected positive regardless of their infection profile.

Infection profiles	Number of plants of each infection profile per sampling years (percentage)					
	2002	2003	2004	2008	2012-2013	2015-2019
TYLCV	0 (0)	6 (30)	5 (10)	7 (7)	1 (11)	0 (0)
TYLCSV	12 (50)	6 (30)	9 (18)	15 (15)	0 (0)	0 (0)
TYLCSV + TYLCV	11 (46)	1 (5)	14 (27)	44 (44)	0 (0)	0 (0)
TYLCV + TYLCV/TYLCSV	0 (0)	2 (10)	5 (10)	1 (1)	6 (67)	12 (44)
TYLCSV + TYLCV/TYLCSV	0 (0)	0 (0)	9 (18)	2 (2)	0 (0)	0 (0)
TYLCSV + TYLCV + TYLCV/TYLCSV	1 (4)	5 (25)	9 (18)	30 (30)	0 (0)	0 (0)
TYLCV/TYLCSV	0 (0)	0 (0)	0 (0)	0 (0)	2 (22)	15 (56)
Total	24 (100)	20 (100)	51 (100)	99 (100)	9 (100)	27 (100)
Total TYLCV/TYLCSV	1 (4)	7 (35)	23 (45)	33 (33)	8 (89)	27 (100)

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