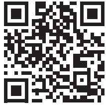

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RESEARCH ARTICLE

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A sensitive real-time RT-PCR reveals a high incidence of *Southern tomato virus* (STV) in Spanish tomato crops

Laura Elvira-González[1], Caterina Carpino[1,2], Ana Alfaro-Fernández[3], María I. Font-San-Ambrosio[3], Rosa Peiró[4], Luis Rubio[1] and Luis Galipienso[1,5]

[1]Instituto Valenciano de Investigaciones Agrarias, Centro de Protección Vegetal y Biotecnología, Ctra. CV-315 Km. 10, 46113 Moncada, Valencia, Spain. [2]University of Palermo, Dept. of Agricultural and Forestry Science, Piazza Marina 61, 90133 Palermo, Italy. [3]Universitat Politècnica de València, Grupo de Virología, Instituto Agroforestal Mediterráneo, Cami de Vera s/n, 46022 Valencia, Spain. [4]Universitàt Politècnica de València, Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Cami de Vera s/n, 46022 Valencia, Spain. [5]Universitàt Politècnica de València, Escuela Técnica Superior de Ingeniería Agronómica y del Medio Natural, Depto. de Biotecnología, Cami de Vera s/n, 46022 Valencia, Spain.

Abstract

Southern tomato virus (STV) is a double-stranded RNA (dsRNA) virus belonging to genus *Amalgavirus* (family *Amalgamaviridae*). STV has been detected in tomato plants showing different symptoms although it has not been demonstrated that STV is the causal agent. To study the STV incidence and its pathogenic role, a sensitive and quantitative real-time reverse transcription-polymerase chain reaction assay (RT-qPCR) was developed. The standard curve performed with viral RNA transcripts allowed a wide dynamic range for STV quantitation from 10^4 to 10^{11} copies/ng of total RNA. STV detection by RT-qPCR was 10^2-fold more sensitive than conventional RT-PCR or RT-LAMP and 10^4-fold more sensitive than molecular hybridization. STV was detected in different tomato plant tissues, as well as in the coat and the embryo of individual seeds. Also, viral concentration remained constant over time in leaf tissues of STV-infected tomato plants. Surveys on different tomato fields from Spain revealed that STV was widespread. In addition, the virus was detected in almost every tomato variety and nursery analyzed. STV-infected tomato plants did not show any disease-related symptom suggesting that the virus was not directly the causal agent of any tomato disease. However, there is no information about the STV effect in mixed infections or in abiotic stressed conditions and further studies must be performed to clarify it. The RT-qPCR assay developed in this work could be implemented on sanitation programs in order to limit the virus spread and could be used to study the effect of STV in mix infections or abiotic stressed conditions.

Additional keywords: *Solanum lycopersicum*; *Amalgaviridae*; *Amalgavirus*; persistent viruses; RT-qPCR.

Abbreviations used: BBWV-1 (*Broad bean wilt virus* 1); CMV (*Cucumber mosaic virus*); CP (putative coat protein); Ct (threshold cycle); Dpt (days post-transplant); dsRNA (double strand RNA); MH (molecular hybridization); PepMV (*Pepino mosaic virus*); PVY (*Potato virus* Y); RdRp (RNA polymerase RNA dependent); RT-LAMP (reverse transcription loop-mediated isothermal amplification); RT-PCR (reverse transcription polymerase chain reaction); RT-qPCR (real-time quantitative RT-PCR); STV (*Southern tomato virus*); TMV (*Tobacco mosaic virus*); ToCV (*Tomato chlorosis virus*); ToMV (*Tomato mosaic virus*); ToTV (*Tomato torrado virus*); TSWV (*Tomato spotted wilt virus*); TYLCV (*Tomato yellow leaf curl virus*).

Authors' contributions: LEG designed the primers and the probe, improved the optimal condition of the RT-qPCR and carried out the experiments to detect STV in different plant tissues and in field samples. CC supported the STV detection experiments from tomato field samples and seedling supplied by nurseries. AAF and MIFSA participated in field surveys, plant material collection and STV detection from seedlings. RP performed the statistical analysis of the data. LR and LG designed the experiments and wrote and edited the manuscript as well. All authors read and approved the final manuscript.

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Correspondence should be addressed to Luis Galipienso: galipienso_lui@gva.es

Introduction

Southern tomato virus (STV) is the type member of the new genus *Amalgavirus* (family *Amalgaviridae*) which contains the persistent viruses *Blueberry latent virus*, *Rhododendron virus A* and *Vicia cryptic virus M* (Gandía *et al.*, 2007; Sabanadzovic *et al.*, 2009, 2010; Martin *et al.*, 2011). STV has a small double-stranded RNA (dsRNA) genome of 3.5 kb in length with two overlapping open reading frames, the 5′-proximal encoding for a putative coat protein (CP) and the 3′-proximal encoding for the RNA-dependent RNA-

polymerase (RdRp). STV is transmitted by seed at rates higher than 70% whereas "horizontal" transmission by vectors is unknown (Sabanadzovic *et al.*, 2009).

Phylogenetic analysis of the RdRp genomic region showed that the genus *Amalgavirus* could represent an evolutionary link between the genus *Totivirus* and *Partivirus* (Sabanadzovic *et al.*, 2009). However, viral particles have not been observed in amalgaviruses, unlike totiviruses and partitiviruses, which form icosahedral virions, even though at very low concentrations in the host tissues (Boccardo *et al.*, 1987). However, the putative CP of amalgaviruses shows no significant sequence similarity with those of totiviruses and partitiviruses (Sabanadzovic *et al.*, 2009), but showed structural homologies with viruses of the genus *Tenuivirus*, suggesting that the ancestor of amalgaviruses could have emerged by a recombination event between the RdRp region from a partitivirus and the CP gene from a tenuivirus (Krupovic *et al.*, 2015). Totiviruses and partitiviruses are persistent dsRNA viruses infecting fungi and plants but they do not induce any apparent symptom in their hosts in contraposition to acute viruses which induce diseases in the infected organisms (Roossinck, 2010). Tenuiviruses are single-stranded RNA (negative polarity) viruses which infect plants and invertebrates inducing diseases and economic losses in important crops such as rice and wheat (Falk & Tsai, 1998; Kormelink *et al.*, 2011).

STV was first detected in tomato plants (*Solanum lycopersicum* L.) showing symptoms of stunting, leaf yellowing and discoloration and reduction of fruit size in USA (California and Mississippi) and South-Western Mexico (Sabanadzovic *et al.*, 2009). Recently, STV was detected in tomato plants in Spain, Italy, France, China and Bangladesh showing similar symptoms but including in some cases strong mosaic and deformation on leaves of infected plants (Candresse *et al.*, 2015; Iacono *et al.*, 2015; Padmanabhan *et al.*, 2015a,b; Verbeek *et al.*, 2015). However, information on STV spread and incidence on tomato fields is scarce. Moreover, the role played by STV on symptom development remains unclear because STV has been frequently detected in mixed infections with acute viruses such as *Pepino mosaic virus* (PepMV), *Tomato mosaic virus* (ToMV), *Cucumber mosaic virus* (CMV) or *Tomato chlorosis virus* (ToCV), *Potato virus* Y (PVY), *Tomato yellow leaf curl virus* (TYLCV), *Tomato spotted wilt virus* (TSWV) and *Tomato torrado virus* (ToTV) (Candresse *et al.*, 2015; Iacono *et al.*, 2015; Padmanabhan *et al.*, 2015a,b; Verbeek *et al.*, 2015). Also, STV has been detected in some asymptomatic plants from some tomato cultivars (Alcala-Briseno *et al.*, 2017; Puchades *et al.*, 2017).

To determine the implication of STV (alone or in combination with other viruses) in tomato disorders, carry out epidemiological studies and implement specific measures of disease control is crucial to have methods for specific and sensitive detection as well as accurate quantitation in plant hosts. Antibodies against STV are not available, so currently the virus can be only detected by using molecular techniques such as polymerase chain reaction preceded of a reverse transcription (RT-PCR), RT and isothermal amplification (RT-LAMP) and molecular hybridization (MH) (Sabanadzovic *et al.*, 2009; Elvira-González *et al.*, 2017; Puchades *et al.*, 2017). However, these techniques are not sensitive enough to detect STV in individual seeds and do not provide accurate quantitative data of STV infections. In the present work, we have developed a sensitive real-time quantitative RT-PCR (RT-qPCR) assay with a TaqMan probe which was applied for detection and quantification of STV from different plant tissues, including individual seeds and monitoring viral accumulation over time. This RT-qPCR approach was used to detect the virus from tomato plants in the field, seedlings distributed by nurseries and seeds of different tomato varieties. The pathogenic role of STV in infected tomato plants was evaluated.

Material and methods

Plant material and RNA extraction

Tomato var. Mariana seeds were grown, and seedlings were individually analyzed by dot-blot hybridization (Puchades *et al.*, 2017) to select STV-infected and non-infected tomato plants. The seedlings were also analyzed by conventional RT-PCR to assess the absence of the two main seed-transmitted viruses described in tomato in Spain, PepMV and ToMV (Aramburu & Galipienso, 2005; Alfaro-Fernández *et al.*, 2009). Then, the seedlings were transplanted to individual pots and grown in an insect-proof greenhouse. Symptoms were periodically monitored in STV-infected plants.

Total RNA extracts were obtained by grounding fresh leaf, root and fruit tissue (200 mg) or individual seeds from STV-infected or non- infected tomato plants in a power homogenizer TissueLyser (Qiagen, Hilden, Germany) with liquid nitrogen. Sap extracts were prepared by resuspension in 500 µL of STE (100 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA pH 7.5) and homogenization with a vortex mixer for 2 min. Total RNAs were finally extracted by using a standard protocol based on phenol:chloroform:isoamyl alcohol extraction followed by ethanol precipitation (Ferriol *et al.*, 2011).

Tomato seeds var. Roque were used to test the presence of STV in the seed coat and embryo (Ali & Kobayashi, 2010). Thus, seeds were germinated on

water dampened filter paper in a sterile Petri dish at 28ºC in an incubator for 7–8 days. Germinated embryos were separated carefully from the seed coat by using sterile scalpels, collected in 1.5 mL tubes kept on ice and then, total RNAs were extracted separately from seed coat and embryo.

To study the incidence the STV: i) leaf tissues of individual plants from tomato fields from Valencian Community (Alicante, Valencia and Castellón provinces) and Great Canary Island were randomly collected and total RNAs extracted and analyzed; ii) tomato seedlings from nurseries of Valencian Community were collected and total RNAs extracted from pools of 5 plants were analyzed; and iii) seed of commercial and local tomato varieties were collected and total RNAs extracted from pools of 10 seeds were analyzed.

Primers/TaqMan probe design and RT-qPCR

The complete nucleotide sequences of STV available in the GenBank database (Acc. No. KT438549, EF442780 and EU413670) were retrieved and aligned with the CLUSTAL W algorithm implemented in the program MEGA 4.0 (Kumar *et al.*, 2008). A set of primers STV-F (5'-TGC CTC CCC AGC TGT CA-3') and STV-R (5'-TGC GTT GGG ATA GAG GAG TGA-3') and a positive-sense Taqman probe (5'-CGC AAC AGA GGT AGA GGC AGA GGC C-3') located in the putative CP gene were designed by using the Primer Express (Applied Biosystems). The STV Taqman probe was tagged with the fluorescent dye 6FAM and the quencher TAMRA at the 5´ and 3´ terminal nucleotide sequences, respectively.

One Step RT-qPCR was performed with the One-Step PrimeScript RT-PCR Kit (TaKaRa) in a LightCycler 480 (Roche), following the manufacturer instructions with some minor modifications: Total RNAs extracts (50 ng) were denaturalized in presence of both STV-F and STV-R primers for 5 min at 95ºC. Then, a mix containing the one-step RT-PCR buffer III, 2 U Ex Taq HS, PrimeScript RT Enzyme Mix II and the TaqMan probe was added to a final volume of 20 µL. To optimize primer and probe concentrations that would result in the lowest average threshold cycle (Ct), two different concentrations of primers (0.2 or 0.4 µM) and probe (0.2 or 0.4 µM) were evaluated. The thermal cycling conditions consisted of reverse transcription at 42ºC for 15 min, incubation at 94ºC for 10 s and 40 cycles of 94ºC for 5 s and 60ºC for 20 s. Total RNA extracts of STV-infected and uninfected tomato plants were used as positive and negative RT-qPCR controls, respectively. Also, virus concentration of positive controls were known since it was determined previously in order to determine the reproducibility of Ct values for each RT-qPCR assay.

Preparation of RNA transcripts and standard curves

The template for *in vitro* transcription, used to obtain the standard curves, was synthesized by conventional RT-PCR. Thus, total RNAs (about 50 ng) from STV-infected tomato plants were denatured in presence of 0.8 µM of primer STV-RT7 (identical to STV-R but including a T7 promoter sequence) and dNTPs mix (125 µM each) by heating for 5 min at 95ºC. For RT, a mix containing 100 U of Superscript IV (Invitrogen), 40 U of Ribolock RNase inhibitor (Thermofisher), DTT (10 mM) and Superscript IV buffer, was added to a final volume of 20 µL and incubated for 20 min to 55 ºC followed by inactivation of Superscript IV by incubating at 80ºC for 10 min. PCR was performed by adding 2 µL of the RT product in a 0.2 mL eppendorf tube containing Taq Buffer, $MgCl_2$ (1.5 mM), dNTPs (125 µM of dATP, dTTP, dCTP and dGTP), STV-F and STV-RT7 primers pair (0.5 µM each), sterile water and 1.5 U of Taq DNA polymerase (Invitrogen) to a final volume of 50 µL. The thermal cycling conditions consisted of an initial incubation at 94ºC for 5 min followed by 40 cycles of 94ºC for 30 s, 60ºC for 30 s, 72ºC for 20 s; and finally at 72ºC for 5 min.

Negative-sense transcripts were synthesized from RT-PCR products with the Megascript T7 kit (Ambion), treated twice with RNase-free DNase (Ambion) and purified (Debreczeni *et al.*, 2011). The concentration and purity of the negative-sense RNA transcripts were evaluated by the spectrophotometer Nanodrop 1000 and the integrity by electrophoresis in 3% agarose gels. The picomoles of ssRNA were calculated with the formula: pmol of ssRNA = µg of ssRNA × (106 pg/1 µg) × (1 pmol/340 pg) × (1/Nb), in which 340 is the average molecular weight of a ribonucleotide and Nb the number of bases of the transcript. The Avogadro's constant (6.023×10^{23} molecules/mol) was used to estimate the number of ssRNA copies. To generate external standard curves, 10-fold serial dilutions containing 10^{11}–10^{1} RNA copies were prepared in total RNA extracts (10 ng/µL) from STV non-infected tomato plants and analyzed by RT-qPCR with the designed STV probe. For each dilution, three technical repeats were done and the Ct mean value was calculated.

Statistical analysis

Data on virus titer were statistically analyzed using a mixed model PROC MIXED in the SAS software (SAS Inst., 2003). Plant effect was included as a random effect, whereas time, position or tissue (depending on the analyses) was included as a fixed effect.

Least Square Difference (LSD) was used for mean comparisons. The assumption of normal distribution of data was assessed using the normal probability plot of the residuals and the assumption of homoscedasticity using the Levene's test. In all the analyses a confidence level of 95% was considered.

Results and discussion

RT-qPCR optimization and STV quantitation in different tomato plant tissues and infected seeds

The lowest Ct values (highest sensitivity) were obtained with the combination of 0.2 µM primers/0.2 µM TaqMan probe. Therefore, this combination of probe and primers was used for subsequent experiments. A standard curve was generated by using serial dilutions of transcripts which enabled the absolute quantification of STV in a dynamic range from 104 to 10^{11} copies/ng of total RNAs (Fig 1). The STV standard curve showed a strong linear relationship with a correlation coefficient $R^2 = 0.9912$, a low variation coefficient (<0.5%), as well as high amplification efficiency (>99%). RT-qPCR of serial dilutions of total RNA extracts obtained from STV-infected tomato leaf tissues allowed STV detection of as little as 10^3 RNA copies/ng total RNA, although virus quantitation was not reliable since such low copy number fell off the linear range of the standard curve. The STV quantitation dynamic range and detection sensitivity were similar to reported for other RNA plant viruses such as *Broad bean wilt virus* 1 (BBWV-1), TSWV and PepMV (Ling *et al.*, 2007; Debreczeni *et al.*, 2011; Ferriol *et al.*, 2011). For STV, RT-qPCR was 10^2-fold more sensitive than RT-PCR and RT-LAMP and 10^4-fold more sensitive than molecular hybridization (Elvira-González *et al.*, 2017; Puchades *et al.*, 2017). Also, RT-qPCR showed a very high reproducibility without noticeable Ct changes of STV-infected positive controls. Higher sensitivity of qPCR versus other molecular techniques has been reported for many viruses and hence this procedure is widely used in diagnosis laboratories (Mackay *et al.*, 2002; Bustin *et al.*, 2005; Logan *et al.*, 2009).

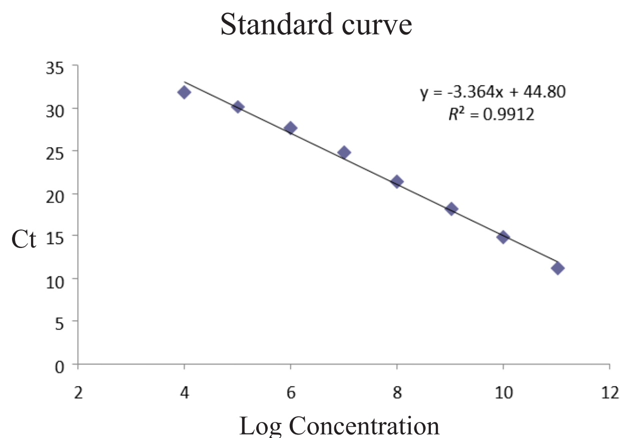

Figure 1. Correlation plot of serially diluted virus RNA transcripts from 10^{11} to 10^4 copies (represented the graphic X axis as Log of nº copies/ng of total RNA) *vs* the average threshold cycle (Ct). Regression equation and correlation coefficient (R^2) values are shown at the top.

STV concentration over time was estimated by RT-qPCR of total RNAs extracts obtained from leaves of five STV-infected tomato plants var. Mariana which were collected at 7, 15, 21, 28 and 35 days post-transplant (dpt) (Fig 2A). STV concentration remained almost constant over time, with mean values ranging from of 1.66×10^7 to 6.94×10^7 copies/ng total RNA. The low variation of STV concentration over time contrasts with acute virus concentration which shows temporal fluctuations depending on the infection stages as consequence of viral replication, uneven virus movement into the plant, and/or activation of the plant defense mechanisms (Ferriol *et al.*, 2011; Tromas *et al.*, 2014). The constant STV concentration over time agrees with the hypothesis of a persistent life cycle in which the virus infects the tomato plants during the complete crop life being transmitted "vertically" to the host offspring via infected seeds (Roossinck, 2010).

Distribution of STV in the plant was studied by RT-qPCR from total RNAs extracts obtained from different tomato tissues such leaves, roots, fruits and seeds (Fig. 2B). For this, five STV-infected tomato plants and twenty seeds of tomato var. Mariana were individually analyzed. STV concentrations were significantly higher in leaf and root tissues (mean values of 2.00 and 2.38×10^7 copies/ng total RNA, respectively) than in fruits and seeds (mean values of 3.26 and 2.62×10^6 copies/ng total RNA, respectively) with p-values <0.05 according to statistical test LSD. However, differences of STV concentration were non-significant (p>0.05) between leaf and root or between seeds and fruit tissues. RT-qPCR enabled STV detection in individual infected seeds which show very high infection rates of 80%, similar to those reported for STV by other authors and much higher than acute seed borne viruses infecting tomato such as ToMV, *Tobacco mosaic virus* (TMV) and PepMV, which had infection rates of about 17, 5 and 0.026 respectively (Hadas *et al.*, 2004; Córdoba-Sellés *et al.*, 2007; Sabanadzovic *et al.*, 2009; Hanssen *et al.*, 2010). Further analysis of STV-infected seeds showed that virus concentration in the embryo (mean value of 2.31×10^6 copies/ng total RNA) was higher than the coat (mean value of 4.24×10^5 copies/ng total

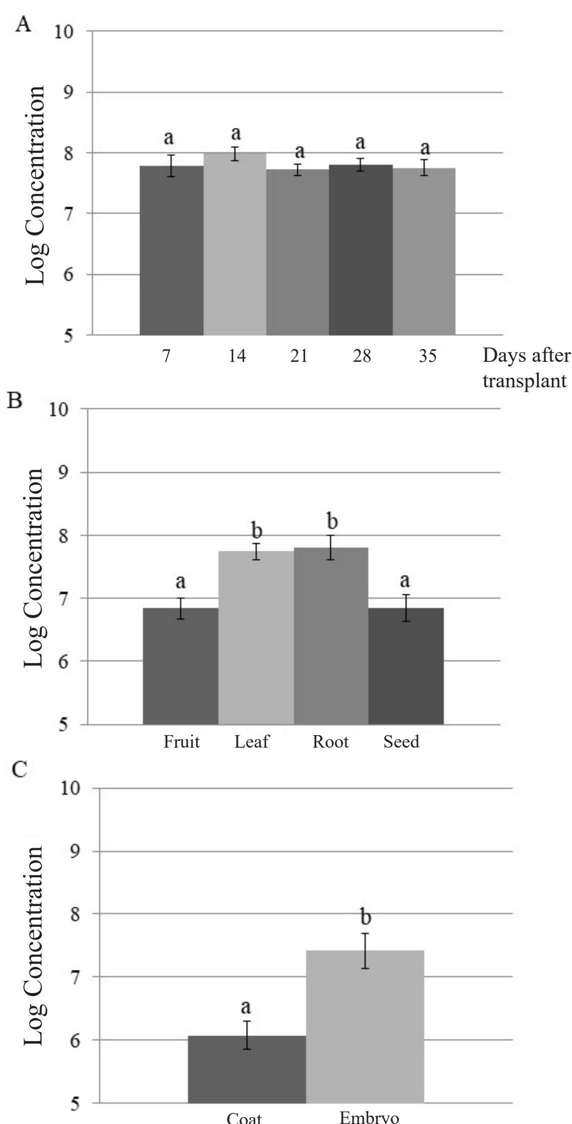

Figure 2. STV quantification over time (A), in different plant tissues (B) and parts of infected seeds (C). Virus concentration values are shown as Log Concentration (nº RNA copies/ng of total RNA) in the Y axis of graphics. Bars represent the standard error of the mean. Letters above the bars indicate significant differences according to a Gamma generalised linear model (overall *p*<0.05 using Bonferroni correction).

RNA) (Fig. 2C). These differences were significant despite the high variation in virus concentrations obtained among different seeds, which ranged from 2.45×10^5 copies/ng total RNA to 9.46×10^8 copies/ng total RNA in the embryo and from 8.87×10^4 copies/ng total RNA to 9.81×10^6 copies/ng total RNA in the coat. This result suggests the direct embryo infection from infected-sexual cells and not by virus invasion from surrounding coat tissues during germination as described for ToMV or PepMV (Sastry, 2013). Embryo infection and high rate of seed transmission are typical of persistent viruses whereas seed-borne acute viruses only infect the seed coat and are transmitted at low rate (Blanc, 2007; Sastry, 2013). High STV concentration in embryo hinders the seed disinfection by physical and chemical treatments (Sastry, 2013).

STV detection in tomato fields, seedlings and seeds of different varieties

To study de incidence the STV in field, leaf tissues of 215 tomato plants of 14 varieties were collected in 14 plots from Alicante, Valencia and Castellón provinces (Valencian Community) and 5 plots from Great Canary Island (Canary Island) (Spain) and total RNAs were extracted (Table 1). STV was detected by RT-qPCR in 9 out of 14 plots (64%) from Valencian Community and in the 5 analyzed plots from Canary Islands (100%). STV incidence was of 51.6% but it varied depending on the tomato production area: 92%

Table 1. STV detection in tomato plots of Valencian Community and Great Canary Island (Spain).

Plot location[1]	Tomato variety[2]	Analyzed samples	Positive for STV
Plot 1 (CP, VC)	Propelians[L]	10	0
Plot 2 (VP, VC)	Valenciano[L]	20	0
Plot 3 (VP, VC)	Montane[L]	9	7
Plot 4 (CP, VC)	Mesapico[C]	15	0
Plot 5 (VP, VC)	Optima[C]	12	0
Plot 6 (VP, VC)	Trujillo[C]	11	2
Plot 7 (VP , VC)	Rosa[C]	9	0
Plot 8 (VP , VC)	Raf[C]	10	5
Plot 9 (AP, VC)	Anaivis[C]	24	22
Plot 10 (AP, VC)	Cherry[C]	10	7
Plot 11 (AP, VC)	Canario[C]	10	7
Plot 12 (AP, VC)	Cherry[C]	11	7
Plot 13 (AP , VC)	Valenciano[L]	7	1
Plot 14 (VP, VC)	Teticabra[L]	7	7
Plot 15 (GCI , CIC)	Tolentina[C]	10	9
Plot 16 (GCI , CIC)	Boludo[C]	10	9
Plot 17 (GCI , CIC)	Boludo[C]	10	10
Plot 18 (GCI , CIC)	Boludo[C]	10	9
Plot 19 (GCI , CIC)	Boludo[C]	10	9
Total	14	215	111

[1]CP, VP and AP: Castellón, Valencia and Alicante provinces, respectively. VC: Valencian Community. GCI: Great Canary Island. CIC: Canary Island Community. [2]L: Local tomato variety generated by owners. C: Commercial tomato variety supplied by seed companies.

in Canary Islands and 39 % in Valencian Community. The STV-infected tomato plants from these fields did not show any symptoms previously reported for STV-infected plants. High incidences are common in persistent viruses such as *Pepper cryptic virus* (genus *Partitivirus*), which asymptomatically infect jalapeño pepper plants (*Capsicum annum* L.) (Arancibia *et al.*, 1995; Sabanadzovic & Valverde, 2011).

To detect STV in tomato seedlings distributed by nurseries, leaf tissues of 32 commercial and 7 local varieties were collected in 39 nurseries from the three provinces of Valencian Community (Alicante, Valencia and Castellón) and total RNAs were extracted (Table 2). Seedlings were analyzed in groups of five plants per each tomato variety. STV was detected by RT-qPCR in 34 out of 39 nurseries (87.2%) from the three provinces of Valencian Community and the virus was detected in 29 out of 32 commercial tomato varieties (90.6%) and in 5 out of 7 local tomato varieties (71.4%). All seedlings showed no symptoms of virus infection. To detect STV

Table 2. STV detection in tomato seedlings distributed by nurseries and seeds from different varieties (commercial and local). Seedlings and seeds were analyzed in groups of 5 plants and 10 seeds, respectively

Genotype[1]	Origin	STV Presence	Genotype[1]	Origin	STV Presence
Amarillo-Rojo[L]	Seed	-	Marinova[C]	Seed	+
Amstrog[C]	Seedling	+	Maties[C]	Seedling	+
Anairis[C]	Seedling	+	Maxifort[C]	Seedling/Seed	+
Angelle[C]	Seed	+	Mini star[C]	Seed	+
Angellety[C]	Seed	+	Montenegro[C]	Seedling	+
Bambelo[C]	Seed	+	Muchamiel[L]	Seedling	+
Barón[C]	Seedling	+	Mutxamiel[L]	Seed	-
Boludo[C]	Seed	+	Olympe[C]	Seedling	+
Brentyla[C]	Seed	+	Optima[C]	Seedling	+
Campillo[L]	Seed	-	Ornela[C]	Seed	+
Cándido[L,C]	Seedling	+	Penjar[L]	Seedling	+
Cofrentes[L]	Seed	-	Pera Caña[L]	Seedling	-
Colgar[L]	Seed	-	Pera de Orihuelo[L]	Seedling	+
Conquita[C]	Seedling	+	Pimiento[L]	Seed	+
Corazón de Buey[L]	Seedling	-	Ramyle[C]	Seedling	+
Dorothy[C]	Seed	+	Realeza[C]	Seed	+
Dumas[C]	Seedling	+	Red Robin[C]	Seedling	-
Ercole[C]	Seedling	+	Red Sky[C]	Seedling	+
Eufrates[C]	Seedling	+	Rio Grande[C]	Seedling	+
Flavoriti[C]	Seedling	+	Robin[C]	Seedling	+
Globe Troter[C]	Seedling	+	Royesta[C]	Seedling	+
Goreti[C]	Seedling	-	Seychelles[C]	Seed	+
Guindo[C]	Seed	+	Shirem[C]	Seed	+
Ibagarza[C]	Seedling	+	Sumer sun[C]	Seed	+
Invictus[C]	Seed	+	Sybilla[C]	Seed	+
Jack[C]	Seedling	+	Top111[C]	Seedling	+
Jupiter[C]	Seedling	+	Tovi Sacro[C]	Seedling	+
Juri[C]	Seedling	-	Tyking[C]	Seedling	+
Katalina[C]	Seed	+	Valenciano[L]	Seedling/Seed	+
Kykuyu[C]	Seedling	+	Vernal[C]	Seed	+
Malpica[C]	Seedling	+	Vilma[C]	Seedling	+
Mariana[C]	Seed	+			

[1]L: local tomato variety generated by owners. C: commercial tomato variety supplied by seed companies.

in tomato seeds, total RNA extracts corresponding to 20 commercial and 7 local tomato varieties were obtained (Table 2). Seeds were analyzed in groups of ten seeds and STV was detected by RT-qPCR in 22 seed lots (81.5%), 20 out of 20 commercial varieties (100%) and 2 out of 7 local varieties (28.6%).

Overall, results reveal a wide dispersion of STV with high incidence in tomato fields from Spain, which could be explained by the wide distribution of this virus on the tomato germplasm. It is remarkable the high number of STV-infected commercial tomato varieties in comparison with the local varieties whose seeds are generated by farmers. None of STV-infected tomato plants showed any disease- related symptom suggesting that STV has not any pathogenic role. This is in concordance with a previous survey carried out in some plots of Valencia province and Great Canary Island (Puchades *et al.*, 2017). Recently, STV has been also detected in symptomless tomato plants var. Sweet Hearts from Florida (Alcala-Briseno *et al.*, 2017). These results suggest that the virus is asymptomatic in tomato, but the role of STV in mixed infections with other tomato viruses remains unknown. Many synergistic interactions among different plant virus combinations have been reported, such as ToTV with PepMV or CMV with different potyviruses, enhancing the disease symptomatology on tomato plants (Hu *et al.*, 1998; Wintermantel, 2005; Murphy & Bowen, 2006; Gómez *et al.*, 2010; Gil-Salas *et al.*, 2012; Chávez-Calvillo *et al.*, 2016). Therefore, further studies must be undertaken to determine possible interactions of STV with other viruses in tomato plants and the effect on plant symptom development. The RT-qPCR procedure developed in this work is a useful tool for sensitive detection of STV in different plant tissues including individual seeds which could be implemented in sanitation programs by seed companies and nurseries. Also, quantitation by RT-qPCR is also useful to study the interactions of STV with other viruses in mixed infections.

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