

Advances on the study of emerging *Southern tomato virus* infecting tomato crops in Mediterranean basin



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Southern tomato virus (STV)



Family *Amalgaviridae*



Southern tomato virus (STV)
Blueberry latent virus (BBLV)
Rhododendron virus A (RhVA)
Vicia cryptic virus M (VCVM)



Persistent viruses

- **Generally do not induce plant symptoms**
- **Seed transmitted with high rates**
- **Low genetic variability**

Southern tomato virus (STV)



Solanum lycopersicum



Double stranded RNA genome (3,4 kb)



Two overlapping coding regions

- 1. Putative coat protein p42 (ORF1)**
- 2. RNA dependent RNA polymerase (POL)**



Vertical transmission by infected seeds (70-80%)
Horizontal transmission by vectors is unknown



Viral particles has been not found

STV Distribution

Mediterranean Basin: France, Spain and Italy (2013-2015)





Why is STV important?






STV was detected in tomato plants showing symptoms of stunting, discoloration and reduction of fruit size

STV role on plant symptoms remains unclear, Why?




-  STV is frequently detected in **mixed infections** with several acute viruses such as *Pepino mosaic virus* (PepMV) or *Tomato mosaic virus* (ToMV)
-  STV was detected in some **asymptomatic tomato plants**



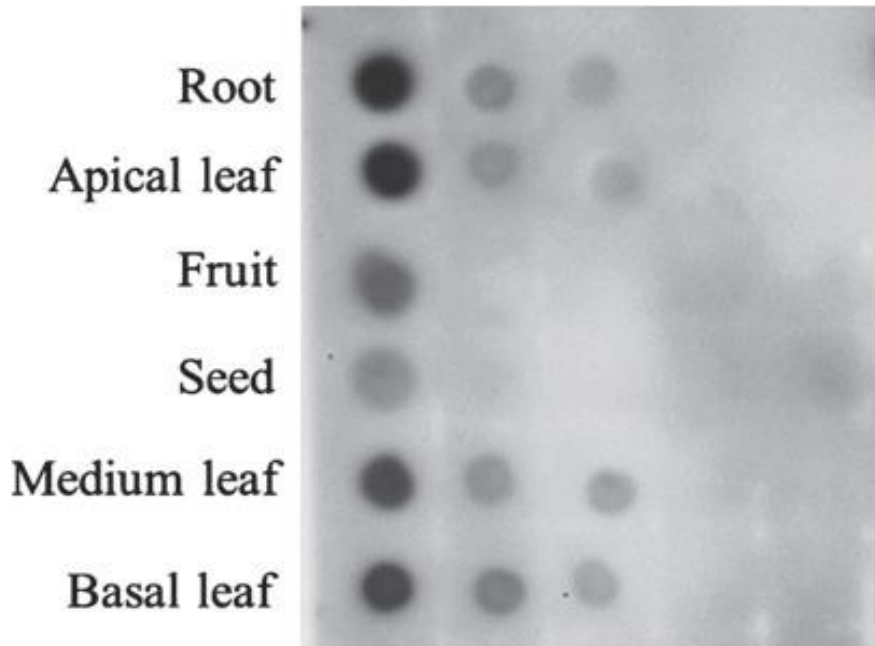
Main Challenges

-  **Implication of STV (alone or in combination with other viruses) in tomato disorders**
-  **Studies of STV epidemiology (Horizontal transmission way)**
-  **To implement specific measures of virus control**

Specific and sensitive methods for STV detection

-  **Molecular hybridization (MH) with a Digoxigenine labelled riboprobe**
-  **(RT-LAMP): one-step transcription loop-mediated isothermal amplification**
-  **(RT-qPCR): One-step real time PCR preceded of a transcription reaction**

Molecular hybridization






Sensitivity: 10^8 copies of STV genome

STV was detected in different plant tissues even in seeds (groups of 5 seeds)

STV was detected from non-processed sap extracts

RT-LAMP

-  **RT-LAMP is a rapid, simple and cost-effective technique:** the amplification reaction can be done in a conventional bath and no expensive thermal cycler is necessary
-  **It is as sensitive as the conventional RT-PCR** and more sensitive than Molecular hybridization
-  **RT-LAMP is less sensitive because of the presence of nucleic acid polymerase inhibitors**

RT-LAMP

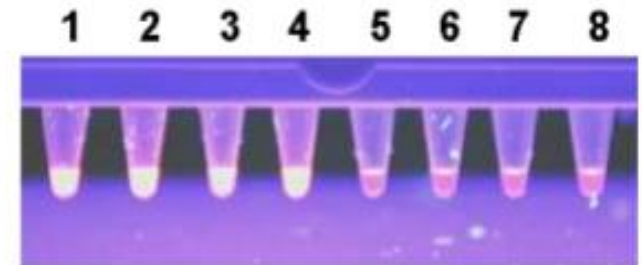
A combination of three pairs of primers were designed in basis of STV genomic sequence



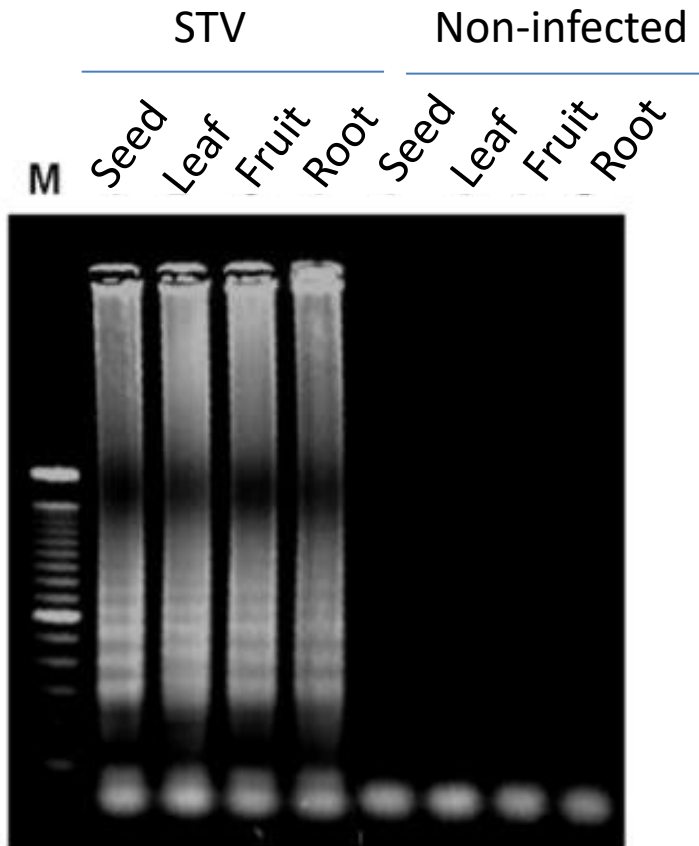
RT-LAMP was done in one step



Amplification products can be visualized by electrophoresis in agarose gels or directly in the reaction tube by adding GelRed under UV



RT-LAMP








Sensitivity: 10^6 copies/ng total RNA

STV was detected in different plant tissues even in seeds (groups of 5 seeds)

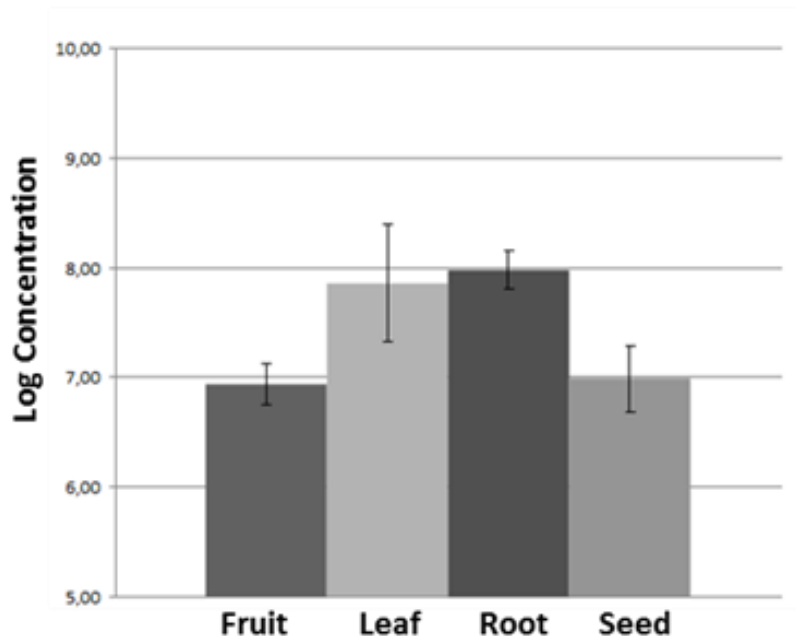
STV was detected from non-processed sap extracts

RT-qPCR

-  **6-FAM Taqman probe**
-  **Sensitivity: 10^3 copies/ng total RNA**
-  **STV was detected and quantified in different plant tissues**
-  **The virus was detected and quantified in individual infected seeds (embryo and coat)**
-  **Virus was quantified over time in infected plants**



STV detection and quantification in different plant tissues



Leaf: $2,00 \times 10^7$ copies/ng of total RNA

Root: $2,38 \times 10^7$ copies/ng of total RNA

Fruit: $3,26 \times 10^6$ copies/ng of total RNA

Seed : $2,62 \times 10^6$ copies/ng of total RNA

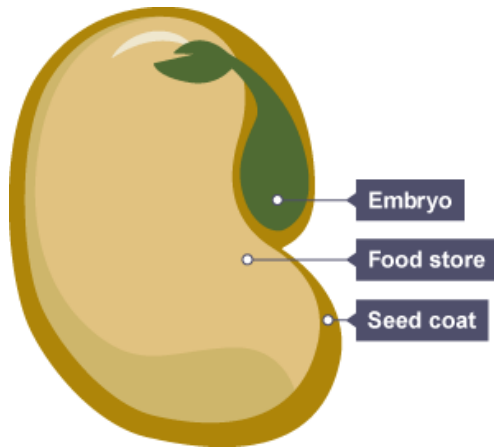
STV concentration was higher in leaf and root than in fruit and seeds



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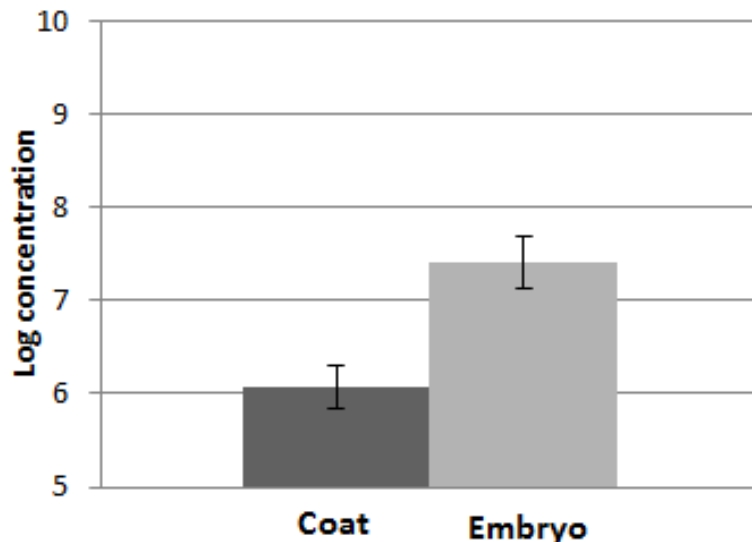
STV detection and quantification in individual seeds



STV was detected in 80% of analyzed seeds

Embryo: $2,31 \times 10^6$ copies/ng of total RNA

Coat: $4,24 \times 10^5$ copies/ng of total RNA



Percentages of STV seed infection were very high and the virus was detected in both embryo and coat

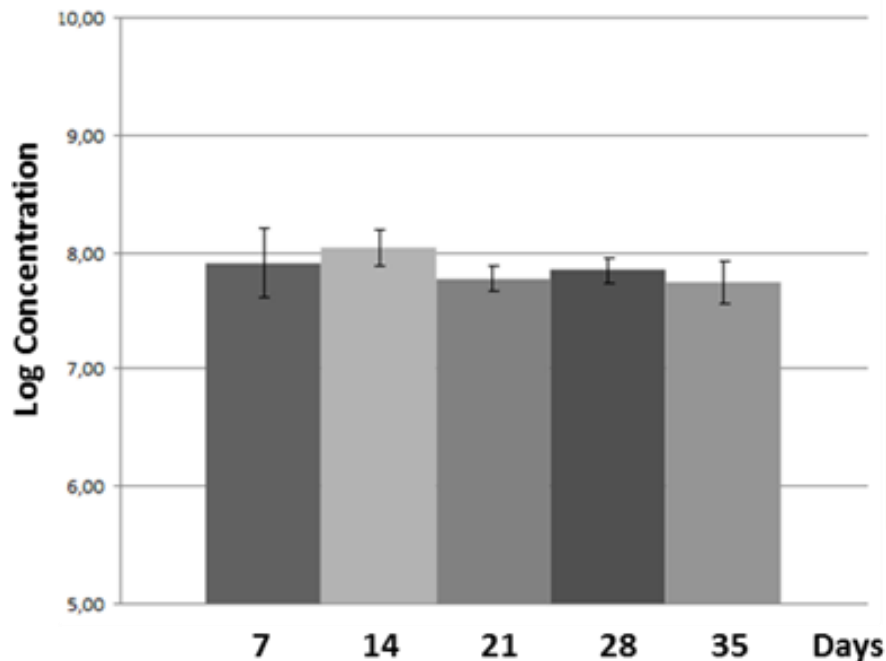
Disinfection of seeds is very difficult



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STV quantification over time



Time 7: $1,66 \times 10^7$ copies/ng of total RNA
Time 14: $6,94 \times 10^7$ copies/ng of total RNA
Time 21: $4,31 \times 10^7$ copies/ng of total RNA
Time 28: $5,16 \times 10^7$ copies/ng of total RNA
Time 35: $4,10 \times 10^7$ copies/ng of total RNA

STV titer remains constant over time





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STV incidence

Seven different plot were analyzed (64 plants):

-  Two Spanish production regions (Canary Islands and Valencian Community)
-  Local (Valenciano y Teticabra) and commercial (Boludo y Tolentina) tomato varieties



STV incidence of 84%

 **57,14% in local tomatoes**

 **92% in commercial tomatoes**

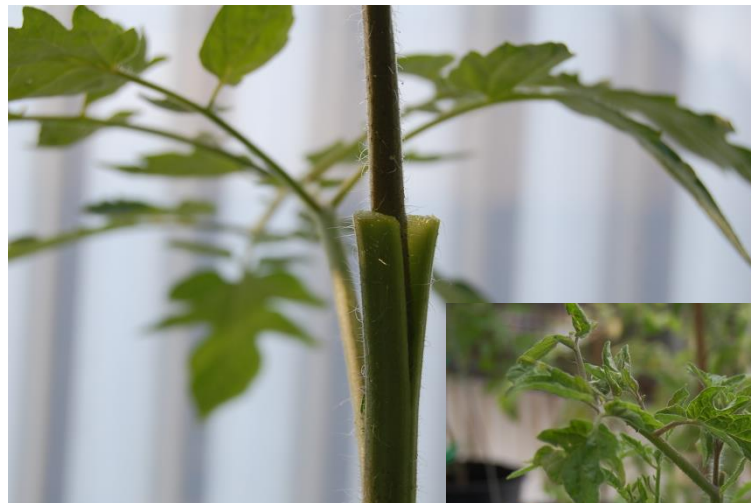
**Different concentrations ($1,03 \times 10^4$ - $8,53 \times 10^9$)
Detected in some asymptomatic tomato plants**

STV transmission assays

Mechanical inoculation



Graft



STV was not transmitted

Production assays



Insect proof green house
14 plants with STV
14 plants without STV

Mariana variety

**The production was
measured**





No significant differences were observed in tomato production between STV- infected and non-infected tomato plants

All the fruits were asymptomatic










Molecular variability studies

GGTAGGGGCAGAGGCCGTGTCACTCCTCTATCCCAACGTAAGGATAGAGGTTGA 767-3
GGTAGAGGCAGAGGCCGTGTCACTCCTCTATCCCAACGCAAGGATAGAGGTTGA 767-4
GGTAGAGGCAGAGGCCGTGTCACTCCTCTATCCCAACGCAAGGATAGAGGTTGA P4499-4
GGTAGAGGCAGAGGCCGTGTCACTCCTCTATCCCAACGCAAGGATAGAGGTTGA P96067-1
GGTAGAGGCAGAGGCCGTGTCACTCCTCTATCCCAACGCAAGGATAGAGGTTGA P96067-2
GGTAGAGGCAGAGGCCGTGTCACTCCTCTATCCCAACGCAAGGATAGAGGTTGA 15331-1
GGTAGGGGCAGAGGCCGTGTCACTCCTCTATCCCAACGCAAGGATAGAGGTTGA

**Putative coat protein gene of different STV isolates was
sequenced**

**Identities among isolates were very high (Nucleotide identity
>99% an Amino Acidic identity of 100%)**

Conclusions

-  **STV was detected in different plant tissues but the higher virus concentrations were found in leaves and roots**
-  **RT-qPCR allowed the virus detection in individual infected seeds (embryo and coat)**
-  **Viral titer remained constant over time**
-  **STV could not be transmitted by graft or mechanical inoculation**
-  **Field surveys showed a high virus incidence (84%)**
-  **STV was detected in some asymptomatic tomato plants**
-  **Virus isolates showed a very low genetic variability**

STV could be a PERSISTENT VIRUS

**STV could not be directly related with tomato
symptomatology**



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Studies in course

- 🍅 Incidence in different production areas (under different conditions and tomato varieties)
- 🍅 Effect of mixed infections with several acute viruses
 - 🍅 Effect under plant stress conditions

Acknowledgments

Work Team (IVIA)

Dr. Luis Rubio
Dr. Luis Galipienso
Laura Elvira (Master student)
Caterina Carpino (PhD student)
Andrés Puchades (Technical support)
Ana Pardo (Technical support)

Work Team (UPV)

Dr. M.I. Font
Dr. A. Alfaro
Esmeralda Sanahuja (Graduate student)

Purificación Benito (Cabildo de gran Canaria)
Juan Moragas (Cabildo de Gran Canaria)
Ana Espino (Gobierno de Gran Canarias)

This work was supported by the INIA project E-RTA2014-00010-C02 co-funded by FEDER 2014-2020 funds