CHARACTERIZATION OF THE TRANSLATIONAL LANDSCAPE OF THE PLANT-VIRUS INTERACTION

Gemma Sans Coll[1,2], Tábata Rosas-Díaz[1,3], Araceli G. Castillo[1,3], Eduardo R. Bejarano*[1,3] and Catharina Merchante*[1,2]

[1]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC),[2] Dpto. Biología Molecular y Bioquímica, UMA, [3] Dpto. Biología Celular, Genética y Fisiología, UMA; Málaga, Spain

1. Introduction

Tomato yellow leaf curl virus (TYLCV) is responsible for a disease which greatly damages tomato crops worldwide (Prasad et al., 2020). It is a member of the *Geminiviridae* family whose genome is composed of a single ssDNA molecule (**Fig. 1**) and, as a virus, requires the host's cellular machinery to infect.

The importance of translation regulation upon infection is critical. However, very little is yet known about the translational changes that occur in the plant in response to the virus. The characterization of the translational landscape of the plant-virus interaction will give meaningful information about key genes related to both the antiviral response of the tomato plant and the virulence strategies of the virus.

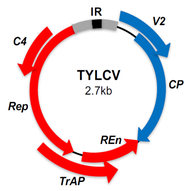
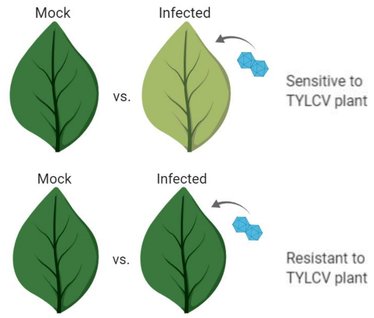


Fig 1. TYLCV genome. In blue, ORFs in the virion chain. In red, ORFs in the complementary chain.

2. Main objectives

- **Obtain the translational landscape of the plant-virus interaction**
- **Functionally characterize tomato genes that are differentially translated upon TYLCV infection**
- **Characterize the translational mechanisms of the TYLCV transcripts in tomato plants.**

3. Determination of the 'transcriptome' and 'translatome' of tomato plants upon TYLCV infection



Systemic response

- RNA-Seq
- Ribo-Seq

Local response

- RNA-Seq through LMD
- TRAP+Ribo-Seq

Fig 2. Schematic of the strategy used to obtain the transcriptome and the translatome of tomato plants upon infection. Plants will be infected with TYLCV using agroinfiltration with *A. tumefaciens* and compared to plants not infected. We will be infecting plants sensitive and resistant to TYLCV. Made in Bio Render.

The pSUC2:HF-SlRPL18B construct has been tested using transitory infiltration on *N. benthamiana* leaves. Polysome profiling and posterior Western Blot with α-Flag on the monosome fraction and the free fraction shows that this fusion protein is incorporated into translating ribosomes, validating our strategy (**Fig. 3**). Transgenic tomato plants are currently being generated containing this construct.

TYLCV is a virus whose replication is restricted to the phloem companion cells (CCs); however, symptoms are observed at a systemic level. To obtain a complete vision of the infection events, we are going to study both the local response and the systemic response. Using a combination of Ribo-seq and TRAP (Translating Ribosome Affinity Purification)-Ribo-Seq using His-Flag-tagged *Solanum lycopersicum* RPL18B under the SUC2 promoter (Truernit et al., 1995), we will be able to collect translating ribosomes specifically in the phloem and compare these footprints with RNA-seq data obtained using laser microdissection (LMD) to compare transcripts actively translating with their total levels (**Fig. 2**). On top of that, we will compare the results between a sensitive and a resistant line to TYLCV.

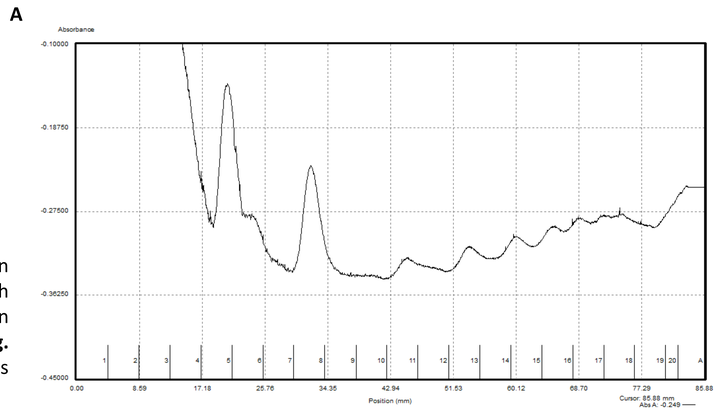


Fig 3. The tagged SlRPL18B is incorporated into translating ribosomes. a) Polysome profile of a transitory HF-SlRPL18B infiltration on N. benthamiana leaves. b) Fractions 4 (free fraction) and fraction 8 (monosome fraction)'s proteins were purified and used in a Western Blot α-Flag.

4. ty-5: tolerant to TYLCV infection

To deepen our knowledge on the regulatory mechanisms of the tomato plant, the analyses of the translatome and transcriptome will be performed on sensitive (Santa Clara) and tolerant (*ty-5* mutant) tomato plant isogenic lines. *Ty-5* contains a recessive mutation located on the *Pelota* gene, which is involved in the recycling phase of the translation cycle (Lapidot et al., 2015).

Although TYLCV infection dynamics have been studied on Moneymaker, they had not yet been described for Santa Clara. Our assays show that Santa Clara exhibits similar symptoms to Moneymaker, with even curler leaves, while *Ty-5* does not show the typical chlorosis, but a slightly decreased leaf size and a slight curl (**Fig. 4**).

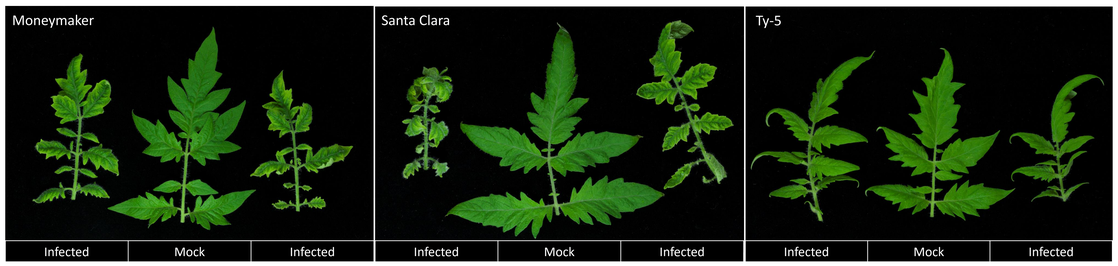


Fig 4. Leaf details from TYLCV infected and mock tomato plants at 27 dpi. Left: Moneymaker. Middle: Santa Clara. Right: Ty-5. Infection was performed using *Agrobacterium tumefaciens* infiltration of the TYLCV genome. Symptoms include yellowing of the leaves (chlorosis), curling and a reduced overall size.

5. Characterization of the cellular machinery involved in the translation of TYLCV

In order to determine the cellular machinery involved the translation of TYLCV, we will be using the MS2-TRAP technology (**Fig. 5**) to bind all proteins that are associated to viral mRNAs.
As TYLCV produces 2 transcripts, we have generated constructs for both, which have been tested using transitory infiltration on *N. benthamiana* leaves and analyzed by fluorescence microscope and Western Blot with α-GFP **(Fig. 6**).

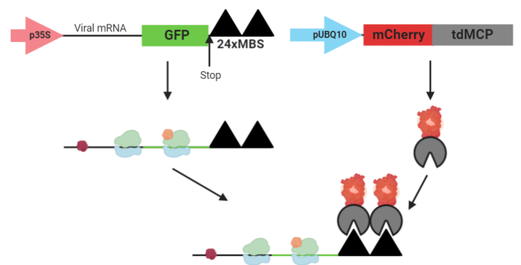
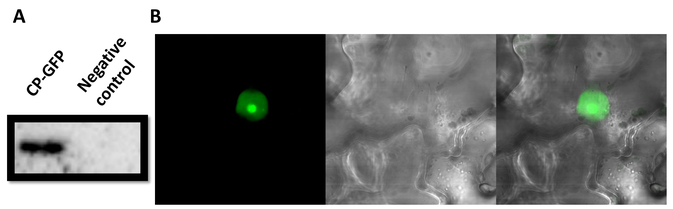


Fig 5. Schematic of the MS2-TRAP technology used to obtain translating ribosomes and its associated proteins. Made in Bio Render.

Fig 6. a) Western blot of *N. benthamiana* leaves transiently expressing the construct carrying the viral mRNA with the 24x MBS b) Fluorescence microscopy of same tissue. The fusion protein CP-GFP can be observed in the nucleus.



6. Expected results

- Find genes differentially translated in infected vs. healthy tomato plants under TYLCV infection.
- Discover key genes important for the TYLCV infection to better understand its virulence strategies.
- Shed light into the role of translation regulation in viral infections.
- Unearth the cellular machinery involved in the translation of TYLCV transcripts and possibly find new viral ORFs.

References

Lapidot, M., Karniel, U., Gelbart, D., Fogel, D., Evenor, D., Kutsher, Y., et al. (2015). A novel route controlling begomovirus resistance by the messenger RNA surveillance factor pelota. PLoS Genet. 11:e1005538.
Prasad A., Sharma N., -Gowthem G., Muthamilarasan M., Prasad M. (2020) Tomato Yellow Leaf Curl Virus: Impact, Challenges, and Management. Trends Plant Sci 2020 Sep;25(9):897-911.
Truernit E, Sauer N. The promoter of the Arabidopsis thaliana SUC2 sucrose-H+ symporter gene directs expression of beta-glucuronidase to the phloem: evidence for phloem loading and unloading by SUC2. Planta. 1995;196(3):564-70.

Acknowledgements

The authors thank Rafael Fernández-Muñoz (IHSM) and Leonardo Boiteaux (EMBRAPA) for sharing the ty-5 and Santa Clara seeds. This work is funded by Grant P18-RT-1218 from the Junta de Andalucía to CM and ERB, and grants BIO2017-82720-P and RYC-2017-1218 to CM.

Presenter: gemmasans@uma.es
*Corresponding authors: Catharina Merchante (merchante@uma.es) and Eduardo R. Bejarano (edu_rodri@uma.es)