CHARACTERIZATION OF THE TRANSLATIONAL LANDSCAPE OF THE PLANT-VIRUS INTERACTION

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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 characterization of translationally regulated genes will give meaningful information about key genes related to both the antiviral response of the tomato plant and the virulence strategies of the virus.

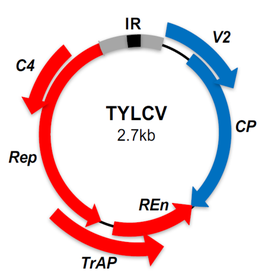

Fig 1. TYLCV genome. In blue, ORFs in the virion chain. In red, ORFs in the complementary chain.

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 host's translational machinery used by TYLCV**

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

TYLCV is a phloem-limited virus that generates systemic symptoms. To study the local and the systemic responses we will follow the strategy shown in Fig. 2. In addition, we will compare the results between a sensitive and a resistant line to TYLCV (see section 4). To achieve this, we have generated the *pSUC2::HF-SlRPL18B* construct that expresses the large subunit ribosomal protein RPL18B under the SUC2 promoter, that is expressed in phloematic tissue. We have already determined that this tagged version of RPL18B is successfully incorporated in translating ribosomes (Fig. 3) and are now transforming tomato plants of the TYLCV-sensitive and resistant genotypes (see point 4).

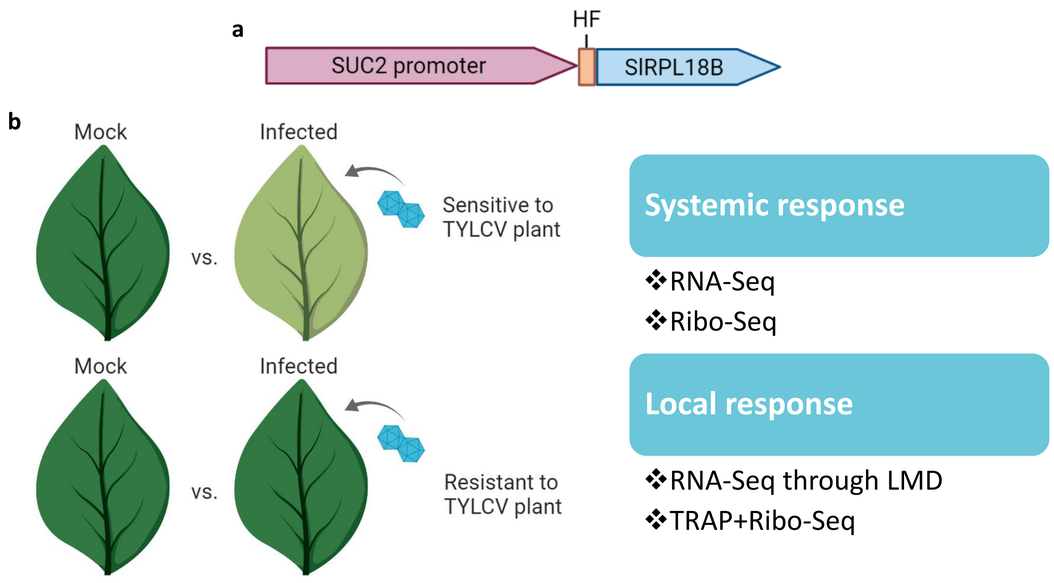

Fig 2. (a) Construct diagram for His-Flag-tagged *Solanum lycopersicum* RPL18B under the SUC2 promoter. (b) Schematic of the strategy used to obtain the transcriptome and the translatome of tomato plants upon infection. Plants will be infected with TYLCV using agroinfiltration with *A. tumefaciens* and compared to uninfected plants. We will be infecting plants sensitive and resistant to TYLCV. Made in Bio Render.

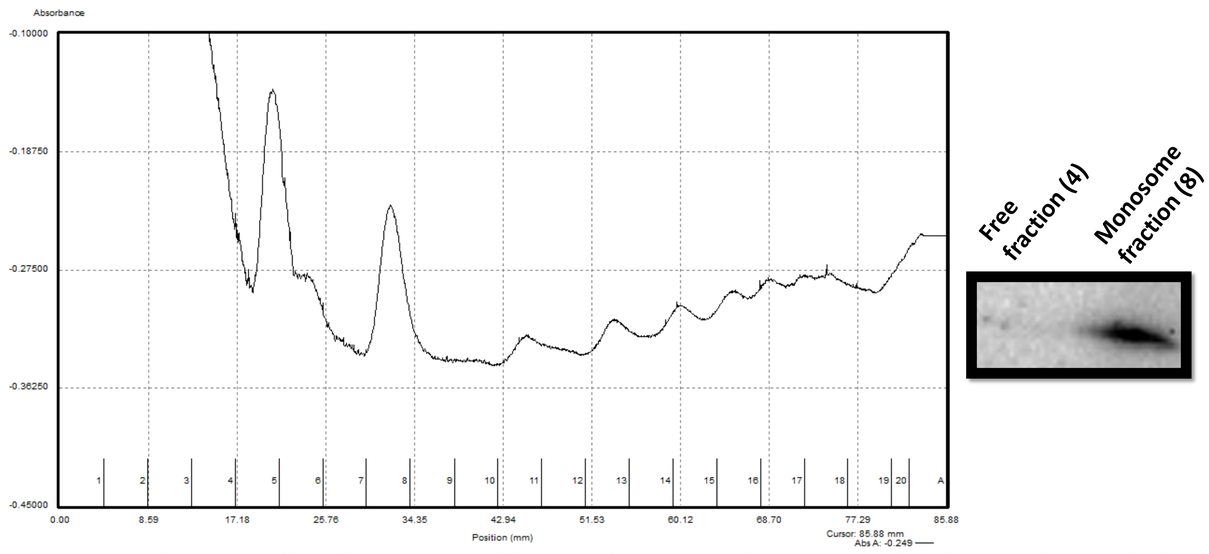

Fig 3. Polysome profiles of a transitory infiltration of pSUC2:HF-SlRPL18B *on N. benthamiana* leaves. The free fraction (4) and monosome fraction's (8) proteins were purified and detected in a Western Blot using α-Flag.

4. ty-5: resistant to TYLCV infection

To deepen our knowledge on the regulatory mechanisms of the tomato plant, the analysis of the translatome and transcriptome will be performed on sensitive (Santa Clara) and resistant (*ty-5* mutant) isogenic tomato lines. *Ty-5* is 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).

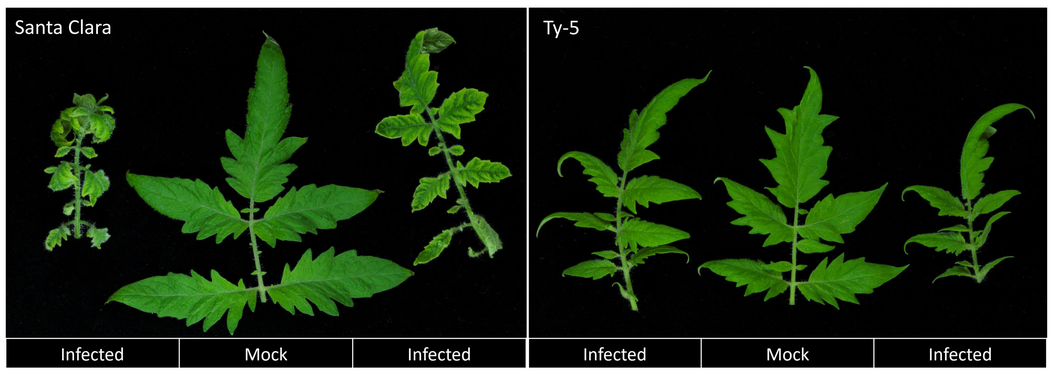

Fig 4. Leaf details from TYLCV infected and mock plants at 27 dpi. Left: Santa Clara. Right: Ty-5. Infection was performed using *Agrobacterium tumefaciens* infiltration of the TYLCV genome. Symptoms include yellowing of the leaves (chlorosis), curling and a reduced overall size.

5. Characterization of the 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 main primary 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).

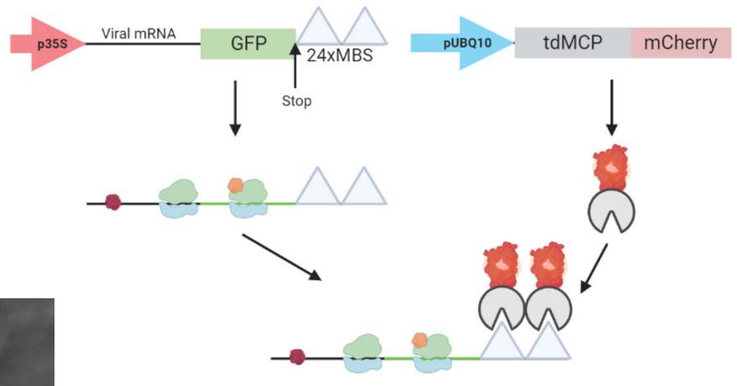

Fig 5. Schematic of the MS2-TRAP technology used to obtain translating ribosomes and its associated proteins. Made in Bio Render.

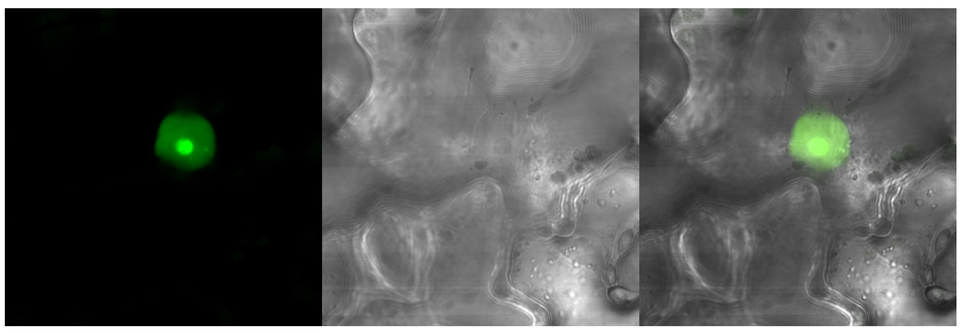

Fig 6. Fluorescence microscope images from *Nicotiana benthamiana* expressing the virion chain transcript. The fusion protein CP-GFP can be observed.

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.

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