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Research article

Transcriptomics of tomato plants infected with TYLCSV or expressing the central TYLCSV Rep protein domain uncover changes impacting pathogen response and senescence

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ABSTRACT

To establish a successful infection viruses need to overcome plant innate immune responses and redirect host gene expression for their multiplication and diffusion. *Tomato yellow leaf curl Sardinia virus* (TYLCSV) is a geminivirus, which causes significant economic losses in tomato. The multifunctional replication associated geminivirus protein (Rep) has an important role during viral infection. In particular, the Rep central domain spanning from aa 120 to 180 is known to interact with viral and host factors. In this study, we used long serial analysis of gene expression to analyse the transcriptional profiles of transgenic tomato plants expressing the first 210 amino acids of TYLCSV Rep (Rep210) and TYLCSV-infected wild-type tomato plants (Wt-Ty). Also, we compared these profiles with those of transgenic Rep130 tomatoes. Comparison of Wt-Ty and Rep210 libraries with the wild-type one identified 118 and 203 differentially expressed genes (DEGs), respectively. Importantly, 55% of Wt-Ty DEGs were in common with Rep210, and no ones showed opposite expression. Conversely, a negligible overlap was found between Rep130 DEGs and Wt-Ty and Rep210 ones. TYLCSV- and Rep210-repressed genes, but not induced ones, overlapped with the leaf senescence process. Interestingly, TYLCSV upregulates expression of genes involved in the negative regulation of programmed cell death (PCD), several of which were also regulated by the abscisic acid. Rep210 upregulated genes related to defence response, immune system processes and negative regulation of PCD. Collectively, our results support a model in which the Rep central domain has a pivotal role in redirecting host plant gene expression.

1. Introduction

To establish a systemic infection a virus needs to overcome more than one plant defence layer and redirect host gene expression for its multiplication and diffusion (Lewsey et al., 2009). RNA silencing, effector-triggered immunity, and pathogen-triggered immunity are three layers of defence that plants have evolved to fight against viruses (Korner et al., 2013; Zvereva and Pooggin, 2012). Compatible plant–virus interactions often induce responses resembling those evocated during basal defence (Wise et al., 2007), whereas incompatible interactions usually culminate in the hypersensitive response (HR), a plant cell death-like phenomenon. Plant programmed cell death (PCD) has a central role in plant–microbe interactions, and an inappropriate PCD can culminate in a susceptible phenotype (Dickman and Fluhr, 2013). Compatible plant–pathogen interactions are those responsible for major economic losses in agriculture.

Tomato yellow leaf curl Sardinia virus (TYLCSV) is a phloem-limited geminivirus possessing a circular single-stranded DNA genome with six genes arranged on both virion- and complementary-sense DNA (Kheyr-Pour et al., 1991). It is responsible, together with other closely related geminiviruses, of one of the world's most destructive tomato diseases (Abhary et al., 2007). Compatible geminivirus infections typically do not show evidence of localized PCD (Hanley-Bowdoin et al., 2013). However, indirect indications suggest that geminiviruses should cope with PCD. In

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fact, *Potato virus X* (PVX) mediated expression of geminiviral proteins can lead to HR (reviewed in Hanley-Bowdoin et al., 2013). Also, some geminiviruses, like TYLCSV, cause leaf chlorosis, a phenotype reminiscent of leaf senescence, which is a type of PCD (Lim et al., 2007).

In the effort to understand how geminiviruses redirect gene expression to accomplish their productive life cycle, the model plant *Arabidopsis thaliana* was initially used as the host (Ascencio-Ibanez et al., 2008; Pierce and Rey, 2013). Transcriptional profiles of plants infected with *Cabbage leaf curl virus* (CabLCV) revealed that CabLCV triggers defence responses via the salicylic acid (SA) pathway, induces genes involved in PCD and alters the expression of genes related to the cell cycle and senescence (Ascencio-Ibanez et al., 2008). Time course analysis of plants infected with *South African cassava mosaic virus* (SACMV) revealed overlaps with CabLCV differentially expressed genes (DEGs) (Pierce and Rey, 2013). However, only 2.3% of SACMV DEGs were continuously expressed across the infection period. More recently, Miozzi et al. (2014) analysed the interaction between TYLCSV and its natural host *Solanum lycopersicon* (cv. Moneymaker) and identified differences in the hormonal signalling and pathways compared to those highlighted in CabLCV and SACMV infections. However, TYLCSV analysis was done using the central portion of leaflet midrib whereas those of CabLCV and SACMV were done on the entire leaf thus preventing a precise comparison.

The multifunctional replication associated geminivirus protein (Rep), which is the only viral protein essential for viral replication, has a modular domain structure (Fondong, 2013). In particular, the region spanning from aa 120 to 180 acts as a multitasking interaction domain binding viral and host factors (Fondong, 2013). This attitude confers to the central region of Rep a pivotal role during geminivirus infections. Of note, the binding of Rep to the plant homologues of retinoblastoma (pRBR) relieves the repression of E2F transcription factors, thus establishing a DNA replication-competent environment (Ascencio-Ibanez et al., 2008; Hanley-Bowdoin et al., 2013). Indirect evidence that the Rep central domain deeply interacts with the host molecular machinery was inferred by the phenotype of transgenic tomato plants expressing different portions of the TYLCSV Rep. In particular, Rep210 tomato plants, expressing the first 210 aa of Rep, showed an altered phenotype (Noris et al., 2004) while those expressing a Rep version lacking the central domain (Rep130) were similar to the wild-type ones (Lucioli et al., 2014). Interestingly, we showed that the transcriptional profiles of Rep130 and wild-type tomato leaves mostly overlap (Lucioli et al., 2014). The above data suggested that Rep130 and Rep210 together with TYLCSV-infected tomato plants could be profitably used to dissect the contribution of the Rep central domain in redirecting host transcription during TYLCSV infection.

In this work, we use long serial analysis of gene expression (LongSAGE) to analyse the transcriptional profiles of TYLCSV-infected wild-type (Wt-Ty) and Rep210 tomato leaflet. The choice to use the entire leaflet and not only the midrib (as done by Miozzi et al., 2014) was based on two reasons: a) plant response to viral infection does not act only in the infected cells; b) we were interested in investigating the role of the central Rep domain in reprogramming host transcription by comparing TYLCSV-infected plants with Rep210 and Rep130 transgenic plants.

We present evidence in support of the notion that TYLCSV should interfere with PCD response to establish a compatible interaction. Moreover, the comparison of Wt-Ty, Rep210 and Rep130 profiles have permitted to appreciate the deep influence exerted by the central Rep domain in TYLCSV-mediated transcriptional reprogramming of host gene expression.

2. Methods

2.1. Rep210 transgenic tomato plants

Transgenic tomato (cv. Moneymaker) plants of line 200 and 201 were previously described in Noris et al. (2004). Western and Southern blot analyses were carried out as described in Lucioli et al.

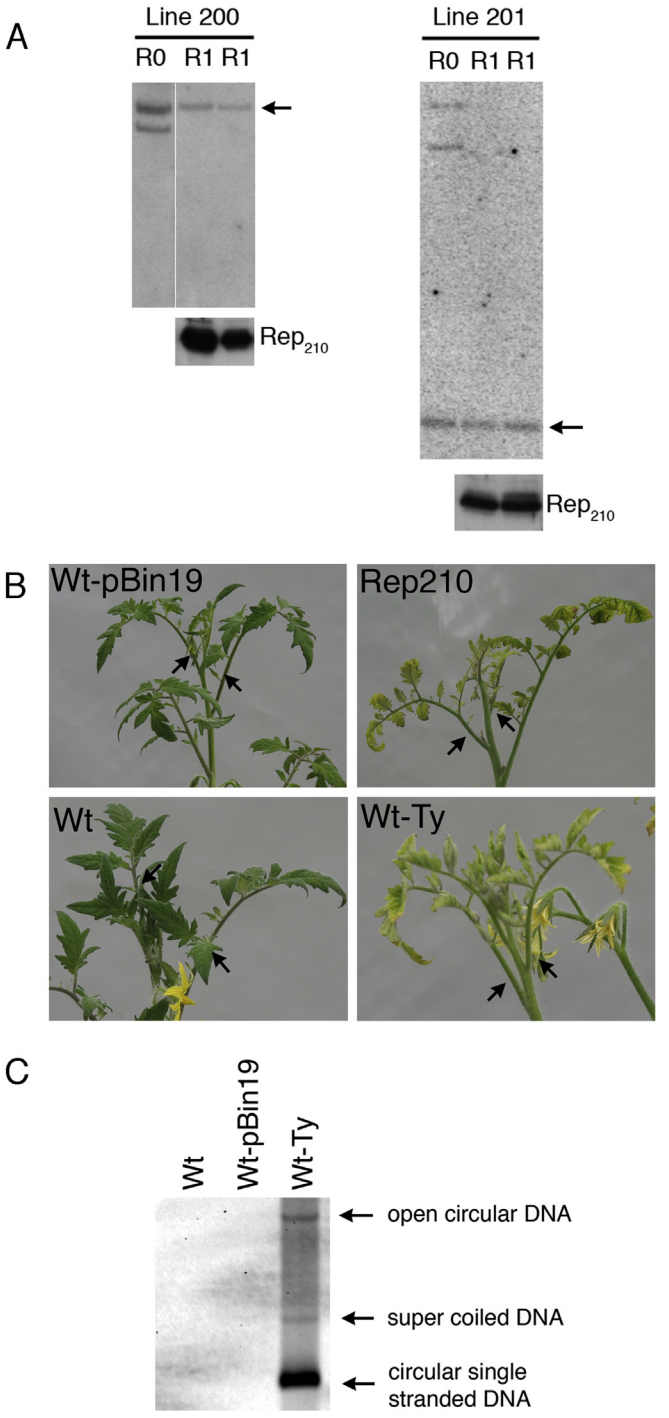

Fig. 1. Characterization of tomato plants used to construct LongSAGE libraries. (A) Southern (upper) and western (lower) blots of Rep210 transgenic lines 200 and 201; arrows indicate the single transgene insertions. (B) Plants used to construct libraries; arrows indicate leaves from which mature leaflets were sampled. (C) Southern blot analysis of TYLCSV replicative forms from indicated samples.