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LONG SERIAL ANALYSIS OF GENE EXPRESSION OF TRANSGENIC PLANTS TO DECIPHER TOMATO YELLOW LEAF CURL SARDINIA VIRUS-TOMATO INTERACTION

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The Tomato Yellow Leaf Curl Sardinia Virus (TYLCSV) is one of the most devastating pathogens that affect tomato (*Solanum lycopersicum*) crops in a vast sub-tropical region of the world. The infecting agent is a ssDNA virus belonging to the family *Geminiviridae*. The viral genome is a 2,7 Kb single stranded circular DNA; two proteins are encoded by the viral strand and four by the complementary one. In order to gain insight into TYLCSV-tomato interactions, our laboratory is currently analysing the transcriptional profile of TYLCSV-infected tomato plants by Long-Serial Analysis of Gene Expression (Long SAGE). Long SAGE identifies 20 nt long sequences (TAGs), allowing the quantitative analysis of transcripts without the need for any prior knowledge of their sequences. 5809 genes were differentially expressed (at least two fold) between healthy and TYLCSV-infected tomato plants (see accompanying poster). To further dissect the viral-host interaction we decided to analyse the transcriptional profile changes induced by the expression of single viral products in transgenic tomato by longSAGE. We focalised our attention on a key factor for viral replication: the replication associated protein (Rep). Rep is a 359aa long protein codified by the viral C1 gene; it is a multifunctional factor able to regulate its own gene transcription and to bind and nick the viral DNA during the initiation and termination phase of the rolling-circle replication (RCR). Moreover, it interacts with plant factors involved in DNA replication and cell cycle regulation, such as the plant retinoblastoma-related proteins (pRBR). We produced different lines of transgenic tomato plants stably expressing two truncated forms of the TYLCSV Rep protein (Rep-210 or Rep-130). Rep-210 posses the DNA binding and cleavage domains and the RBR-binding domain whereas Rep-130 retains only the DNA binding and cleavage domains. Both Rep-210 and Rep-130 have the ability to down regulate C1 gene transcription binding the C1 gene promoter. Rep-130 and Rep-210 plants were grown alongside healthy and TYLCSV-infected wt tomato under controlled conditions in three independent experiments. Rep-210 and Rep-130 longSAGE libraries were produced and are currently being sequenced. Transcriptome analysis of Rep-210 and Rep-130 plants will be presented.