Tomato yellow leaf curl Sardinia virus-resistant tomato plants expressing the multifunctional N-terminal domain of the replication-associated protein show transcriptional changes resembling stress-related responses

ALESSANDRA LUCIOLI¹, ALESSANDRA BERARDI¹, FRANCESCA GATTI^{1,†}, RAFFAELA TAVAZZA¹, DANIELE PIZZICHINI^{2,‡} AND MARIO TAVAZZA^{1,*}

¹Agenzia Nazionale per le Nuove Tecnologie, l'Energia e l'Ambiente (ENEA), UTAGRI-INN, C.R. Casaccia, Via Anguillarese 301, 00123 Rome, Italy

²Biogen srl, c/o C.R. Casaccia, Via Anguillarese 301, 00123 Rome, Italy

SUMMARY

The N-terminal domain (amino acids 1–130) of the replication-associated protein (Rep₁₃₀) of *Tomato yellow leaf curl Sardinia virus* (TYLCSV) retains the ability of full-length Rep to localize to the nucleus and to down-regulate C1 transcription when ectopically expressed in plants, both functions being required to inhibit homologous viral replication. In this study, we analysed the effect of Rep₁₃₀ expression on virus resistance and the plant transcriptome in the natural and agronomically important host species of TYLCSV, *Solanum lycopersicum*. Tomato plants accumulating high levels of Rep₁₃₀ were generated and proved to be resistant to TYLCSV. Using an *in vitro* assay, we showed that plant-expressed Rep₁₃₀ also retains the catalytic activity of Rep, thus supporting the notion that this protein domain is fully functional. Interestingly, Rep₁₃₀-expressing tomatoes were characterized by an altered transcriptional profile resembling stress-related responses. Notably, the serine-type protease inhibitor (Ser-PI) category was over-represented among the 20 up-regulated genes. The involvement of Rep₁₃₀ in the alteration of host mRNA steady-state levels was confirmed using a distinct set of virus-resistant transgenic tomato plants expressing the same TYLCSV Rep₁₃₀, but from a different, synthetic, gene. Eight genes were found to be up-regulated in both types of transgenic tomato and two encoded Ser-PIs. Four of these eight genes were also up-regulated in TYLCSV-infected wild-type tomato plants. Implications with regard to the ability of this Rep domain to interfere with viral infections and to alter the host transcriptome are discussed.

INTRODUCTION

Geminiviruses are a large family of plant viruses possessing a genome of one or two circular single-stranded DNA (cssDNA) molecules packaged within twin-shaped capsids (Jeske, 2009).

They infect a range of economically important crop species in tropical and subtropical regions, thus representing a major threat to agriculture in these geographical areas. The family *Geminiviridae* is divided into four genera on the basis of genome arrangement, host range and insect vector (Fauquet *et al.*, 2008). *Tomato yellow leaf curl Sardinia virus* (TYLCSV) belongs to the genus *Begomovirus*, possesses a monopartite genome, infects dicot plants, is transmitted by the aleurodide *Bemisia tabaci* and is responsible, together with other closely related begomoviruses, for one of the most destructive tomato diseases (Moriones and Navas-Castillo, 2000).

Geminiviruses replicate in the nuclei through a combination of rolling-circle and recombination-mediated replication (Preiss and Jeske, 2003; Saunders *et al.*, 1991; Stenger *et al.*, 1991). The replication-associated protein (Rep), which is encoded by the C1 gene (also called AL1 and AC1), is the only protein that is essential for replication (Elmer *et al.*, 1988). Rep executes its functions through the combined action of three macro-domains, namely the N-terminal, central and C-terminal domains.

The N-terminal region consists of approximately the first 130 amino acids (Rep₁₃₀) and is involved in several tasks. This protein domain is sufficient for the initiation and termination of rolling-circle replication. It introduces a site-specific nick in the conserved nonanucleotide sequence TAATATT_{AC}, which is present on the plus strand of viral DNA remaining covalently linked to the 5' phosphate (Laufs *et al.*, 1995). In addition, this domain mediates the binding of Rep to viral double-stranded DNA (dsDNA) sequences, known as iterons, which act as specificity determinants of the interaction (Arguello-Astorga *et al.*, 1994; Fontes *et al.*, 1994). Rep binding to iteron sequences is required for viral replication (Fontes *et al.*, 1994) and mediates the inhibition of its gene transcription (Eagle *et al.*, 1994).

In the case of TYLCSV, plant-expressed Rep₁₃₀ retains the ability to be compartmentalized within the nucleus/nucleolus and to inhibit C1 gene transcription, whereas a further deletion of 10 amino acids from its C-terminus abolishes both functions (Lucioli *et al.*, 2003; Sardo *et al.*, 2011). In addition, a recent deletion analysis conducted on the Rep of *Tomato golden mosaic virus* (TGMV), a bipartite begomovirus, has shown that the Rep region

*Correspondence: Email: mario.tavazza@enea.it

Present addresses: [†]Dipartimento Fisiologia Generale, Università del Salento, Lecce, Italy;

[‡]Genelab Srl, via Roma 26, 96010 Palazzolo Acreide (Siracusa), Italy.

responsible for the binding to the small ubiquitin-related modifier (SUMO)-conjugating enzyme (SCE1) lies inside this N-terminal domain between amino acids 85 and 114 (Sánchez-Durán *et al.*, 2011). SCE1 is the enzyme that conjugates SUMO polypeptide to the target proteins (Saracco *et al.*, 2007).

The central part of Rep, which spans approximately from amino acids 120 to 180, acts as a multitasking interactor domain mediating Rep self-oligomerization (Clerot and Bernardi, 2006; Orozco *et al.*, 2000) as well as Rep binding to viral products, such as C3/AL3 (Settlage *et al.*, 2001) and the coat protein (CP) (Malik *et al.*, 2005), or to plant cellular factors, such as proliferating cell nuclear antigen (PCNA) (Bagewadi *et al.*, 2004) and homologues of the retinoblastoma family (pRBR) (Kong *et al.*, 2000). Rep-pRBR binding plays a fundamental role in begomovirus infection by relieving the repression of E2F transcription factors, thus establishing a DNA replication-competent environment (Ascencio-Ibanez *et al.*, 2008; Egelkrout *et al.*, 2001, 2002; Hanley-Bowdoin *et al.*, 2004).

The Rep C-terminal domain extends beyond amino acid 180 to the C-terminus and contains ATP-binding and ATP hydrolysis motifs. When expressed alone, this protein domain retains ATPase activity, whereas, for helicase activity, both the oligomerization and C-terminal domains need to contribute (Choudhury *et al.*, 2006).

Transgenic expression of viral sequences has been used extensively to obtain virus-resistant plants (Prins *et al.*, 2008). In a previous study, we reported that *Nicotiana benthamiana* plants transformed with a TYLCSV sequence capable of encoding Rep₁₃₀ only, as a result of mutation of the C4 start codon (Lucioli *et al.*, 2003), showed resistance to the homologous virus (Lucioli *et al.*, 2008). Large amounts of the Rep₁₃₀ protein expressed from a single copy transgene are required to confer long-lasting resistance. Rep₁₃₀ acts as a dominant negative mutant tightly inhibiting TYLCSV C1 gene transcription and virus replication in protoplasts and plants (Lucioli *et al.*, 2003, 2008). Transgenic plants that do not accumulate high levels of Rep₁₃₀ do not impair viral replication efficiently and the initial interference is overcome with time as a result of the ability of the replicating TYLCSV to silence the Rep₁₃₀ transgene (*Rep130*) (Lucioli *et al.*, 2008).

In some instances, the ectopic expression of geminiviral proteins has been shown to have an impact on the plant transcriptome, thus highlighting additional aspects of their involvement in viral infection (Lai *et al.*, 2009; Lozano-Duran *et al.*, 2012; Selth *et al.*, 2005; Trinks *et al.*, 2005). With regard to Rep, the transgenic expression of TGMV AL1 highlights its involvement in the induction of PCNA transcription in terminally differentiated cells (Nagar *et al.*, 1995), transcriptional activation most probably being the result of Rep-pRBR binding, which, in turn, relieves the repression of E2F transcription factors (Ascencio-Ibanez *et al.*, 2008; Egelkrout *et al.*, 2001, 2002; Kong *et al.*, 2000). The modulation of host transcription by Rep has also

been highlighted by the up-regulation of 162 genes following the transfection of *Arabidopsis thaliana* protoplasts with *Mungbean yellow mosaic virus* AC1 (Trinks *et al.*, 2005).

From a biotechnological point of view, an ideal interference product should be able to confer resistance without altering plant physiology. Here, we extended our previous studies conducted on the model plant *N. benthamiana* by analysing the ability of Rep₁₃₀ to confer TYLCSV resistance in its natural host, tomato. We showed that tomato-expressed Rep₁₃₀ retains the ability to confer high levels of resistance to TYLCSV and to recognize and cut the conserved nonanucleotide sequence. Importantly, TYLCSV-resistant Rep₁₃₀-expressing transgenic tomato plants have an altered transcriptional profile resembling a stress-related response, which is also characterized by the up-regulation of the serine-type protease inhibitors (Ser-PIs) that are known to be induced by various damage stimuli (Turrà and Lorito, 2011). The implications of the ability of this domain to interfere with viral infection and to alter host transcription are discussed within the framework of virus resistance and of the possible additional role for this Rep macro-domain in viral infection.

RESULTS

Rep₁₃₀-expressing tomato plants are resistant to TYLCSV

Tomato was transformed with the *Rep130* transgene previously used to produce transgenic *N. benthamiana* plants resistant to TYLCSV (Lucioli *et al.*, 2003, 2008). Nineteen transgenic plants were obtained, and 10 expressed Rep₁₃₀. None of the Rep₁₃₀-expressing T0 plants showed a compatible segregation with a single locus insertion. Plants expressing high levels of Rep₁₃₀ were identified in the progeny of lines 403, 406 and 410 (Fig. S1A, see Supporting Information). Selected T1 plants of these lines were characterized by Southern blot analysis. A single hybridizing DNA fragment was detected in T1 410-8, 410-11 and 406-27 plants highly expressing Rep₁₃₀ (Fig. 1A,B). T2 410-11 and 406-27 transgenic plants showed a slightly altered phenotype consisting of a less pronounced leaflet serration (Fig. S2A, see Supporting Information).

Virus resistance was initially evaluated by challenging nine T2 406-27 transgenic tomato plants with TYLCSV. Rep₁₃₀-expressing plants were virus free until the end of the experiment [8 weeks post-inoculation (wpi)] as evaluated by dot blot analysis, whereas the three segregants and the four wild-type plants were all infected between 2 and 3 wpi (Fig. S1B). The virus-resistant phenotype of T2 406-27 plants was confirmed in a second resistance test, in which 10 challenged transgenic plants remained TYLCSV free until 11 wpi. Similarly, only two of the 10 T2 410-11 plants were infected at 11 wpi. The multilocus line 403, which did not accumulate large amounts of Rep₁₃₀ in all the T1 plants (Fig. S1A

Fig. 1 Molecular characterization of tomato T1 Rep₁₃₀ transgenic lines. (A) Western blot analysis of protein extracts from T1 generation of transformants 410, 406 and 403. Numbers above the lanes correspond to single T1 transgenic plants. NT, nontransgenic segregant plants. (B) Southern blot of *Hind*III-digested DNA from T1 transgenic plants as in (A), probed with a Rep-specific digoxigenin-labelled transcript. WT, wild-type tomato plants. (C) Western blot analysis of protein extracts from T1 generation of transformant 403 at 0 and 8 weeks post-inoculation (wpi). S, susceptible plant; R, resistant plant.

and data not shown), showed a complex virus-resistance trait. Two of the 17 T1 Rep₁₃₀-expressing plants were already infected at 2 wpi, eight became infected later on and seven remained virus free until 8 wpi, whereas all the segregants and the 10 wild-type plants were infected between 2 and 3 wpi.

To ascertain whether virus susceptibility was linked to the reduction in Rep₁₃₀ accumulation, as reported previously in *N. benthamiana* plants (Lucioli *et al.*, 2008), Western blot analysis was conducted at both 0 and 8 wpi. In TYLCSV-infected plants, but not in resistant T1 403 plants, a drastic reduction in Rep₁₃₀ accumulation was observed at 8 wpi (Fig. 1C). Thus, tomato plants with a single copy transgene insertion and highly expressing Rep₁₃₀ were resistant to TYLCSV, as reported previously for *N. benthamiana* (Lucioli *et al.*, 2008).

Ectopically expressed Rep₁₃₀ retains the ability to recognize and cut the conserved geminivirus nonanucleotide sequence

In a previous study, we showed that Rep₁₃₀, when ectopically expressed in *N. benthamiana* plants, retains some of the biochemi-

cal and biological functions described for the full-length Rep. In particular, Rep₁₃₀ was shown to tightly repress TYLCSV C1 gene transcription (Lucioli *et al.*, 2003) and to be specifically targeted to the nucleus (Sardo *et al.*, 2011), both functions being essential for its ability to inhibit TYLCSV replication in single cells and whole plants (Lucioli *et al.*, 2003; Sardo *et al.*, 2011). In addition, the ability of TYLCSV Rep₁₃₀ to inhibit TYLCSV replication, but not that of the Portuguese strain of *Tomato yellow leaf curl virus* TYLCV-Mld (AF105975) (Lucioli *et al.*, 2003), suggests that either Rep₁₃₀ is inactive in recognizing and cutting the conserved nonanucleotide sequence of geminiviruses or that this activity is not able to confer TYLCV-Mld resistance. In this study, to further characterize the functionality of Rep₁₃₀ expressed in tomato plants and to extend its functional relation with that expressed in *N. benthamiana*, protein extracts of *N. benthamiana* line 308 (Lucioli *et al.*, 2003, 2008), as well as those of line T2 410-11, were analysed in terms of their ability to recognize and cut *in vitro* the oligonucleotide gem12 (Fig. 2A), which comprises the conserved nonanucleotide sequence of geminiviruses. If Rep₁₃₀ cleaves this substrate, a protein–nucleotide adduct consisting of Rep₁₃₀ bound to the new 5' extremity generated by the cutting activity is expected (Laufs *et al.*, 1995). In the case of gem12, the adduct should contain Rep₁₃₀ linked to the last five nucleotides (ACCGG) (Fig. 2A), and thus with a reduced mobility on sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE). A band migrating more slowly than Rep₁₃₀ was detected in both transgenic, but not wild-type, *N. benthamiana* and tomato extracts incubated with gem12. This additional band was not observed when Rep₁₃₀ extracts were incubated with the unrelated oligo pJIT60 (Fig. 2B).

To confirm that the reduced mobility of Rep₁₃₀ was caused by the generation of the expected adduct, two additional oligonucleotides, gem3-12 and gem12-3, were used. gem3-12 and gem12-3 are both substrates for Rep₁₃₀, but differ from gem12 in that they have three additional nucleotides at their 5' or 3' end, respectively (Fig. 2A). However, gem12-3, but not gem3-12, is expected to generate a protein–nucleotide adduct migrating more slowly than that observed for gem12. Thus, when Rep₁₃₀ tomato extracts were incubated with gem3-12, gem12 or gem12-3, the predicted adducts were generated (Fig. 2C). The above data support the notion that Rep₁₃₀, accumulating in TYLCSV-resistant transgenic *N. benthamiana* and tomato plants, retains the ability of the wild-type Rep to recognize and cut the nonanucleotide sequence.

Rep₁₃₀-expressing tomato plants show transcriptional changes resembling defence-related responses

As we were interested in evaluating the feasibility of using Rep₁₃₀ as a biotechnological tool to confer TYLCSV resistance, we decided to analyse the potential impact, if any, of ectopically expressed Rep₁₃₀ on the tomato transcriptome. Two Long Serial Analysis of

Fig. 2 Analysis of *in vitro* cleavage activity of Rep₁₃₀ expressed in transgenic *Nicotiana benthamiana* and *Solanum lycopersicum* plants. (A) Oligonucleotides used as substrates for *in vitro* cleavage assay; the arrow indicates the cleavage site; the conserved nonanucleotide sequence is boxed. (B, C) Western blot analysis of protein extracts from wild-type (WT) or Rep130 transgenic *N. benthamiana* (T1 308) and *S. lycopersicum* (T2 410-11) plants incubated for 30 min at 37 °C with different concentrations of oligonucleotides, as reported above each lane.

Gene Expression (LongSAGE) libraries were constructed from leaves of wild-type and a pool of T2 406-27 and T2 410-11 plants; 1521 wild-type and 1327 Rep130 clones were sequenced; 34 005 wild-type and 26 869 Rep130 tags were obtained and 94% had an overall quality value (QV) of ≥ 40 . Of the 13 267 wild-type and 11 506 Rep130 unique tags, 4264 and 3489, respectively, had abundance levels of two or greater (Table 1). Collectively, 60 874 high-quality sequence tags, accounting for 7008 unique *bona fide* transcripts (tag frequency ≥ 2), were identified. Statistical analysis identified 32 tags differentially represented ($P < 0.01$, chi-squared 2×2 test; fold $\geq |2|$) between the two libraries. Of these, 25 identified a unique tomato chromosomal location, four had multiple matches on the genome, two identified transgene derived

Table 1 Frequency of unique tags in wild-type (WT) and Rep130 Long Serial Analysis of Gene Expression (LongSAGE) libraries.

Tag abundance	Unique tags	
	WT	Rep130
All	13 267	11 506
>10	354	255
7–10	293	253
5–6	433	310
4	398	334
3	805	643
2	1 981	1 694

sequences and one did not result in any match (Table S1, see Supporting Information). Of the 29 tags associated with the tomato genome, three identified potential antisense transcripts, whereas, of the four tags with multiple genomic matches (Table S1), only one was associated with more than one gene, identifying the Ser-PI gene family to which *Pin1* belongs (Table 2). In total, in Rep₁₃₀-expressing plants, 20 and six sense transcripts were up- and down-regulated, respectively, in comparison with the wild-type transcripts (Table 2).

Gene ontology (GO) term enrichment analysis using the Plant MetGenMap database (Joung *et al.*, 2009) identified five functional-related categories in the up-regulated gene set, all containing the same five genes (Table S2, see Supporting Information). The functional Ser-PI category was the most statistically significant with $P = 3.28e^{-10}$, <0.001 and 0 as evaluated by Bonferroni, simulation and false discovery rate (FDR) statistical tests, respectively (Table S2).

To validate the LongSAGE transcriptional data, a semi-quantitative reverse transcription-polymerase chain reaction (sqRT-PCR) analysis was performed on the five Ser-PI genes identified in the GO enrichment analysis and on the two other members of the *Pin1* gene family (Table 2). As shown in Fig. 3, all the seven genes analysed were up-regulated in Rep₁₃₀-expressing plants, thus supporting both LongSAGE and GO enrichment data. The identification of a highly statistically significantly enriched GO category in such a restricted number of genes suggests that the up-regulation of Ser-PIs plays a functional role.

In our study, to determine whether the 26 genes identified by LongSAGE analysis (Table 2) were similarly regulated under different stimuli, microarray expression data of *Solanum lycopersicum* and *A. thaliana* leaf tissues were analysed using BiMax clustering analysis at the Genevestigator web-based interface (Hruz *et al.*, 2008). This analysis identified a set of five genes in *A. thaliana* which were co-up-regulated in eight of the 396 conditions (perturbations) analysed (Fig. 4 and Table S3, see Supporting Information). Of note, three of the five co-up-regulated genes belong to the class of Ser-PI (Fig. 4). The eight conditions were related to inoculation with plant nonpathogenic (*PsthrC⁻* and *PsthrpA*), nonpathogenic on *A. thaliana* (*Pph*) and avirulent (*PstAvrB*) bac-

Table 2 Differentially expressed genes in transgenic Rep130 tomato plants.

Tag sequence	Tag frequency		Fold*	P value†	Unigene	Definition	Rep130‡
	WT	Rep130					
ttgtctgaagaatt	1	10	12.7	0.002	SGN-U314020	CCR4-NOT complex subunit 7	No
tgatttgacaaaac	2	19	12	0	SGN-U314484	Defensin-like protein	Yes
taagatctgtttgtt	1	8	10.1	0.007	SGN-U315457	Prohibitin	No
gtttcttataaact	0	8	10.1	0.001	SGN-U317358	AAA-type ATPase protein	Yes
tagaatctattgttaa	0	6	7.6	0.006	SGN-U323251	CER3	Yes
tgggactgtgttaga	0	6	7.6	0.006	SGN-U320067	4-Coumarate CoA ligase	No
gctgtgctcaccatta	2	10	6.3	0.006	SGN-U317269	Membrane protein	No
cctactcgttacaccc	2	10	6.3	0.006	SGN-U313689	60S ribosomal protein L27	No
tactcttactatata	9	40	5.6	0	SGN-U312824	Proteinase inhibitor (PIN1 family)	No
					SGN-U312825	Proteinase inhibitor (PIN1 family)	No
					SGN-U312826	Proteinase inhibitor (PIN1 family)	No
cttcaacctcggatcg	4	17	5.4	0.001	Solyc01g007320.2.1	H ⁺ -transporting two-sector ATPase	No
cacagagcatttagc	5	20	5.1	0	Not found	—	—
tcataaagaataagt	3	11	4.6	0.009	SGN-U313578	ATP citrate lyase subunit	No
ctaactattataatgt	3	11	4.6	0.009	SGN-U312588	Proteinase inhibitor I20 (PIN2)	Yes
tctttctaataaaat	8	26	4.1	0	SGN-U312623	Proteinase inhibitor I3	No
gagcaacgagtcacaa	4	13	4.1	0.007	SGN-U312362	Tetraspanin 8	Yes
tttgatctagttaaaa	6	16	3.4	0.007	SGN-U315041	Histone H2A.F/Z	No
gttttctaataaact	10	26	3.3	0.001	SGN-U312622	Proteinase inhibitor I3	Yes
tttggtttcattcata	14	32	2.9	0.001	SGN-U313267	Glycine-rich protein	No
aatgaaaatttccagt	14	29	2.6	0.002	SGN-U315396	Histone H2B	Yes
tggtgtatttttattt	15	29	2.4	0.004	SGN-U314266	Defensin	No
tttagcgaatttgaa	23	38	2.1	0.004	SGN-U312624	DNAJ chaperone	Yes
ctggggtgacaatttt	12	1	−9.5	0.008	SGN-U313641	Malate dehydrogenase	No
taggcattatagcatc	21	2	−8.3	0.001	SGN-U312914	40S ribosomal protein S5	No
tatttttccgattct	9	0	−7.1	0.008	SGN-U330102	Prolyl endopeptidase	No
tcctccaggcctctt	9	0	−7.1	0.008	SGN-U312776	40S ribosomal protein S25	No
aaaattttatgttgcg	19	4	−3.8	0.01	SGN-U315059	Tropinone reductase I	No
ggttttacgcaactaag	110	33	−2.6	0	SGN-U314750	Chlorophyll <i>a-b</i> -binding protein 13	No
ttcctcctgagaagca§	2	10	6.3	0.0062	SGN-U312809	Cysteine proteinase	na
gatctccgatcgggaa§	9	0	−7.1	0.0077	SGN-U322540	Uncharacterized mitochondrial protein	na
tatccgcttgctggtg§	25	6	−3.3	0.0054	SGN-U312927	Cobalamin-independent methionine synthase	na¶

*Ratio between Rep130 and wild-type (WT) tags normalized for the size of the Serial Analysis of Gene Expression (SAGE) libraries; only genes with fold > |2| were considered.

†Chi-squared 2 × 2 statistical test (http://telethon.bio.unipd.it/bioinfo/IDEG6_form/).

‡Genes differentially expressed in Rep130syn tomato plants according to semi-quantitative reverse transcription-polymerase chain reaction (sqRT-PCR) analysis.

§Tags that identify potentially antisense transcripts.

¶Not analysed.

terial strains of *Pseudomonas syringae* (Kemmerling *et al.*, 2007; Thilmony *et al.*, 2006; Yun *et al.*, 2011), as well as infiltration with some pathogen-associated molecular patterns (PAMPs), such as the flagellin-derived 22-amino-acid peptide (flg22) and Hairpin Z (HrpZ). The most extended overlaps (seven co-up-regulated genes) were found in the *A. thaliana* Col-0 and in the gain-of-function mutant for 5-nitrosogluthathione (GSNO) reductase (*atgsnor1-1*), both inoculated with *PstAvrB*, and in HrpZ-treated plants. In addition, in *PstAvrB/atgsnor1-1*, *PsthrpA/Col-0*, *HrpZ/Col-0*, *PstAvrB/Col-0*, *PstAvrB/sid2* and *PsthrpC/Col-0*, between one and three genes were also co-down-regulated (Table S3).

Overall, this analysis suggests that ectopic expression of Rep₁₃₀ evokes a response which is similar to that observed during attempted bacterial infections. However, there could be other reasons apart from Rep₁₃₀ expression to explain the variation in

host gene expression observed in transgenic plant lines 410-11 and 406-27. To ascertain the involvement of Rep₁₃₀ in the altered transcriptional profile observed, we used tomato plants (M. Tavazza, unpublished data) transformed with a synthetic Rep₁₃₀ transgene (*Rep130syn*) (Lucioli *et al.*, 2008). *Rep130syn* encodes the same Rep₁₃₀ protein as that encoded by the *Rep130* transgene, although the two transgenes differ by more than 15% in terms of nucleotides as a result of the use of synonymous codons (Lucioli *et al.*, 2008). We assumed that, if Rep₁₃₀ expression was responsible for an altered host gene expression, some of the 26 genes differentially expressed in plant lines 410-11 and 406-27 should also be similarly regulated in transgenic Rep130syn tomato plants.

Two Rep130syn lines, 860 and 867, were used, showing a 3:1 segregation ratio and accumulating Rep₁₃₀ at a level comparable with lines 410-11 and 406-27. Similar to that observed with lines

Fig. 3 Semi-quantitative reverse transcription-polymerase chain reaction (sqRT-PCR) on serine-type endopeptidase inhibitor genes up-regulated in Rep130 transgenic tomato plants. Ethidium bromide-stained agarose electrophoretic gels of sqRT-PCR products from wild-type (WT) or Rep130 transgenic tomato plants as indicated above the lanes. Left panels, specific unigenes as listed; right panels, reference gene actin SGN-U313632.

Fig. 4 BiMax clustering of *Arabidopsis thaliana* genes homologous to Rep130 transgenic tomato up-regulated genes. The selected bicluster (red box) represents five genes co-regulated in eight conditions. *Arabidopsis thaliana* genes homologous to tomato unigenes are indicated above the box, tomato unigenes are reported in parentheses, perturbations are reported on the left and asterisks indicate protease inhibitor genes. The *A. thaliana* homologues of tomato SGN-U314484 (defensin-like), SGN-U315457 (prohibitin 1-like), SGN-U317269 (membrane protein) and SGN-U312623 (protease inhibitor I3) are not represented on the *A. thaliana* ATH1 array.

Fig. 5 Tomato genes co-regulated in Rep130 and Rep130syn transgenic tomato plants. Ethidium bromide-stained agarose electrophoretic gels of semi-quantitative reverse transcription-polymerase chain reaction (sqRT-PCR) products from wild-type (WT) or Rep130syn transgenic tomato plants, as indicated above the lanes. Left panels, specific unigenes as listed; right panels, reference gene actin SGN-U313632.

410-11 and 406-27, all 10 T1 860 transgenic plants challenged with TYLCV remained virus free until the end of the experiment (11 wpi). In addition, protein extracts of T1 Rep₁₃₀-expressing plant lines 860 and 867 were able to recognize and cut *in vitro* the oligonucleotides gem12 and gem12-3 (Fig. S3, see Supporting Information). Similar to 410-11 and 406-27 transgenic plants, Rep130syn lines 860 and 867 were characterized by a slightly altered phenotype (Fig. S2B). Thus, lines 860 and 867 closely resembled lines 410-11 and 406-27 from a phenotypic and functional point of view.

sqRT-PCR analysis underlined that eight of the 26 genes differentially expressed in Rep130 plants were similarly regulated in Rep130syn plants (Table 2 and Fig. 5). The eight tomato genes were annotated as follows: the tetraspanin8 (*TET8*; SGN-312362), one AAA-type ATPase (SGN-U317358), one DnaJ-like (SGN-U312624), one histone H2B (SGN-U315396), the Eceriferum 3 (*CER3*, SGN-U323251), one defensin-like (SGN-U314484) and two Ser-PIs, the tomato protease inhibitor II (*Pin2*; SGN-U312588) and one protease inhibitor of Kunitz-type (SGN-312622). Again, of the eight genes, GO enrichment analysis identified Ser-PI as significantly ($P < 0.01$) enriched (Table S4, see Supporting Information).

BiMax clustering analysis was used to identify at least three co-regulated genes, and identified 15 perturbations in which *TET8*

Fig. 6 Semi-quantitative reverse transcription-polymerase chain reaction (sqRT-PCR) analysis, in *Tomato yellow leaf curl Sardinia virus* (TYLCSV)-infected tomato plants, of the core set of genes co-regulated in Rep₁₃₀ and Rep₁₃₀syn transgenic plants. Ethidium bromide-stained agarose electrophoretic gels of sqRT-PCR products from wild-type (WT), pBIN19- or TYLCSV-agroinoculated tomato plants, as indicated above the lanes. Left panels, specific unigenes as listed; right panels, reference gene actin SGN-U313632.

and the two Ser-PIs were co-up-regulated ($P < 0.05$) (Fig. S4, see Supporting Information). The identified perturbations mostly overlapped, as observed in Rep₁₃₀ lines, with those related to attempted bacterial infections. Thus, using two distinct groups of TYLCSV-resistant Rep₁₃₀-expressing plants, we were able to identify a core of eight genes whose mRNA steady-state levels appear to increase through the ectopic expression of Rep₁₃₀.

A subset of Rep₁₃₀ up-regulated genes is induced in TYLCSV-infected tomatoes

The eight genes induced by the ectopic expression of Rep₁₃₀ could also be potentially regulated following TYLCSV infection. To test this hypothesis, sqRT-PCR analysis of the eight genes was performed on virus-infected wild-type tomato plants (at 4 wpi), which were grown alongside the Rep₁₃₀ plants used to build the LongSAGE libraries. In addition, to distinguish between genes regulated by TYLCSV and those potentially regulated in response to the virus agroinoculation procedure, sqRT-PCR analysis was also performed on tomato plants agroinoculated with the empty vector pBIN19. As shown in Fig. 6, *TET8* (SGN-312362), AAA-type ATPase (SGN-U317358), histone H2B (SGN-U315396) and DnaJ-like (SGN-U312624) genes were up-regulated during TYLCSV infection.

However, CER3, the defensin-like protein and two Ser-PIs were not. Importantly, none of the eight genes appeared to be differentially regulated in tomato plants agroinoculated with pBIN19 (Fig. 6). Thus, four of the eight genes were co-regulated in Rep₁₃₀-expressing and TYLCSV-infected tomato plants.

DISCUSSION

In this study, we broadened our understanding of the biochemical functions of plant-expressed Rep₁₃₀ and its ability to confer resistance to TYLCSV in the agronomically important tomato crop by focusing on the transcriptome of Rep₁₃₀ transgenic, TYLCSV-resistant, tomato plants. Using two different sets of transgenic plants, both expressing the same Rep₁₃₀, but with transgenes that differed by more than 15% in the coding sequence, we showed that Rep₁₃₀ confers resistance to TYLCSV, whilst also being responsible for transcriptional changes that resemble stress-related responses. Eight host genes increased mRNA steady-state levels and four were also induced by TYLCSV. We believe that our findings raise new questions with regard to the possible involvement of this Rep domain in viral infection and the feasibility of this resistance strategy.

One protein, two genes, two plant species: Rep₁₃₀ consistently confers TYLCSV resistance

Virus resistance tests reveal that, in tomato, as in *N. benthamiana*, the expression of a large amount of Rep₁₃₀ from a single copy transgene confers resistance to TYLCSV, regardless of the coding sequence. The similarity between the model plant and tomato is further evident in the behaviour of the transgenic lines that show partial resistance to TYLCSV. Indeed, in these lines, TYLCSV infection causes a drastic reduction in Rep₁₃₀ irrespective of the plant species studied (Fig. 1C; Lucioli *et al.*, 2008). In the model plant, shutdown in protein expression is a result of virus-induced transgene silencing which does not induce a recovery phenotype (Lucioli *et al.*, 2008). Similarly, we did not observe a recovery phenotype in TYLCSV-infected T1 403 tomato plants.

In addition, the equivalence of the two plant systems is further reinforced by the biochemical behaviour of plant-derived Rep₁₃₀. *In vitro* experiments clearly indicate that Rep₁₃₀ from transgenic *N. benthamiana* and tomato plants retains the ability to specifically recognize and cut the conserved geminivirus sequence, thus remaining covalently linked to the new 5' extremity (Fig. 2). Similar results were also obtained with protein extracts from Rep₁₃₀syn tomatoes (Fig. S3), confirming the equivalence of the proteins synthesized from the two different transgenes.

The fact that plant-expressed Rep₁₃₀ retains cutting activity leads to further questions on how such cutting is involved in resistance. With regard to homologous virus resistance, further experiments will be required to address this question. Here,

however, in the light of the above results, we can now re-analyse previous data on the inability of TYLCSV Rep₁₃₀ to inhibit heterologous TYLCV-Mld replication (Lucioli *et al.*, 2003).

There are various hypotheses to explain this result. First, the TYLCV-Mld nonamer sequence may not be accessible to Rep₁₃₀ *in vivo*. It has been demonstrated that, *in vitro*, TYLCSV Rep is able to cut ssDNA, but not dsDNA, and it has been proposed that, during rolling-circle replication, the nonamer sequence is melted or stabilized as a cruciform extrusion by Rep before cutting (Laufs *et al.*, 1995). Interestingly, in the *Mungbean yellow mosaic India virus*, *in vitro* Rep binding causes DNA distortion, which melts the origin of DNA replication (Singh *et al.*, 2008). Similarly, in the case of the small staphylococcal plasmid pT181, cruciform extrusion is mediated by the binding of the plasmid-coded RepC (Noirot *et al.*, 1990). If *in vivo* Rep binding is a prerequisite for the extrusion of the ssDNA nonamer sequence, the inability of TYLCSV Rep₁₃₀ to inhibit TYLCV-Mld replication can be easily reconciled with the strain-specific recognition of the iterons (Arguello-Astorga and Ruiz-Medrano, 2001). Alternatively, if cruciform extrusion *in vivo* does not require direct or indirect interaction of Rep, the lack of inhibition of TYLCV-Mld replication could be explained by supposing that Rep₁₃₀ does not work as a dominant negative mutant. However, this hypothesis seems unlikely; indeed, Rep₁₃₀ lacks functional domains that are essential for ATPase (Desbiez *et al.*, 1995) and helicase (Clerot and Bernardi, 2006) activities, as well as for the binding of viral and host factors (Castillo *et al.*, 2003).

TYLCSV-resistant tomato plants expressing Rep₁₃₀ show transcriptional changes characterized by the induction of Ser-PI genes and partially overlapping with those evoked by attempted bacterial infections

Our finding that plant-expressed Rep₁₃₀ retains TYLCSV Rep functions associated with this domain and confers effective resistance to the homologous virus in tomato plants led us to explore its impact on the plant transcriptome with the dual aim of performing an initial molecular transgenic assessment and to evaluate whether transcriptional changes evoked by Rep₁₃₀ could be observed in TYLCSV-infected plants. We thus decided to cross-validate the LongSAGE data obtained on Rep130 lines 410-11 and 406-27 by analysing which of the 26 differentially expressed genes (Table 2) were also similarly regulated in Rep130syn lines 860 and 867. The two sets of transgenic plants compared were not only derived from independent transformation experiments and possessed different transgene sequences, but were also produced and grown in different years. This experimental design was used to maximize the discovery of *bona fide* Rep₁₃₀-regulated genes, and managed to identify eight genes with increased mRNA steady-state levels (Fig. 5). Four of these, namely DnaJ-like, *TET8*, AAA-type ATPase and histone H2B, were also up-regulated in TYLCSV-infected plants (Fig. 6). Of note, in *Cabbage leaf curl virus*

(CaLCuV)-infected *A. thaliana* plants, the homologues of DnaJ-like (At3G44110; AtJ3), *TET8* (At2G23810), AAA-type ATPase (AT3G28510) and the Ser-PI of Kunitz-type (AT1G17860) are also up-regulated (Ascencio-Ibanez *et al.*, 2008). Thus, four of the eight genes are up-regulated by TYLCSV and CaLCuV and three (*TET8*, AAA-type ATPase and DnaJ-like) have the two geminivirus infections in common.

The induction of a common set of genes in Rep₁₃₀-expressing and TYLCSV-infected tomato plants, together with the overlap observed with CaLCuV infection, suggests an additional role for this domain in modulating host transcription; moreover, this is probably not the result of its ability to bind dsDNA. Indeed, TYLCSV iteron sequences differ from those of CaLCuV, as well as from several other tomato-infecting geminiviruses (Arguello-Astorga and Ruiz-Medrano, 2001).

Interestingly, co-immunoprecipitation of *A. thaliana* RBR interaction partners identifies, among others, AtJ3 (Gutzat, 2009), which is possibly an interactor of TYLCSV C3 (Lozano-Duran *et al.*, 2011). The above findings, together with the evidence that Rep interacts with both RBR and C3 (Kong *et al.*, 2000; Settlege *et al.*, 1996), suggests that Rep-mediated DnaJ-like up-regulation could be useful for a productive geminivirus infection. However, in *A. thaliana*, in addition to mediating different biological functions through protein–protein interaction (Shen *et al.*, 2011; Yang *et al.*, 2010), AtJ3 is thought to be part of an Hsp70:J-protein machine required to chaperone the correct folding of proteins. Thus, its up-regulation in Rep₁₃₀-expressing plants, as well as in TYLCSV- and CaLCuV-infected ones, could be related to the chaperone activity (Rajan and D'Silva, 2009).

TET8 is a member of the tetraspanin protein superfamily, which is present in mammals, insects, fungi and plants (Wang *et al.*, 2012). In mammals, tetraspanins are involved in the regulation of several processes, including bacterial and viral infections (Hemler, 2008). In plants, apart from TET1, which plays a role during cell differentiation and organ formation in meristems, the biological role of the other tetraspanins is still unclear (Wang *et al.*, 2012).

Meta-analysis of the *A. thaliana* genes regulated on infection with CaLCuV and seven distinct (+)ssRNA viruses shows that, although the expression of 5296 genes is altered by at least one of the eight viruses, no one gene is similarly regulated in all conditions, and only seven are co-up-regulated by six viruses (including CaLCuV) (Rodrigo *et al.*, 2012). One of these seven is the AAA-type ATPase, which, in this study, was revealed to be up-regulated in TYLCSV-infected and Rep₁₃₀-expressing tomato plants. No biological information is available for AT3G28510, whereas, for AATP1, one of its closest *A. thaliana* homologues, a role as a negative regulator of stress-related genes has been suggested (Rama Devi *et al.*, 2006). Accordingly, the tobacco homologue NtAAA1 is involved in the attenuation of the hypersensitive response (Lee and Sano, 2007a, 2007b). We do not know the

biological function of this tomato AAA-type ATPase. However, the up-regulation of its *A. thaliana* homologue in six different viral infections, together with the roles played by AATP1 and NtAAA1 proteins, suggests its potential involvement in the attenuation of cellular responses to a perceived stress.

The hypothesis that Rep₁₃₀ is perceived as a stress is further supported by BiMax clustering analyses conducted on genes up-regulated in Rep130 and Rep130syn plants (Figs 4 and S4). These analyses highlight the similarities to the plant responses evoked by attempted bacterial infections and PAMP/microbe-associated molecular pattern (MAMP) treatments. Interestingly, the thread of Rep₁₃₀-expressing tomato plants is the up-regulation of the Ser-PIs. *Pin2* is known to be induced by a range of physical stimuli (Graham *et al.*, 1986; Green and Ryan, 1972; Herde *et al.*, 1995; Roux *et al.*, 2006), as well as by bacterial infections sustained by *Pseudomonas syringae* pv. *tomato* (*Pst*) (Pautot *et al.*, 1991). Systemin and jasmonic acid (JA) are some of the primary intracellular wound signals triggering *Pin2* expression (Orozco-Cárdenas *et al.*, 2001; Sun *et al.*, 2011), whereas H₂O₂ acts as a second messenger (Orozco-Cárdenas *et al.*, 2001).

To rule out the possibility that the over-representation of Ser-PI mRNAs was the result of unnoticed mechanical damage, we inspected LongSAGE libraries for the presence of tags identifying genes known to be induced by wounding. Tags for early wound response genes, such as allene oxide synthase, lipoxygenase, prosystemin and polygalacturonase catalytic subunit, as well as tags for two late wound response genes, the serine-type carboxypeptidase (Moura *et al.*, 2001) and leucine aminopeptidase (Pautot *et al.*, 1993), were all present in the LongSAGE libraries and did not show any statistical changes (Table S5 see Supporting Information).

The induction of *Pin2* in Rep₁₃₀-expressing plants independent of wound-related damage suggests an involvement for this Ser-PI beyond the well-established role in protecting plants against herbivorous insects. Of note, salicylic acid (SA) and flg22 treatments, as well as attempted (*Pst*hrc⁻ and *Pst*AvrB), but not productive, *Pst* infections induce the accumulation of H₂O₂ (Angel Torres, 2010; Chen *et al.*, 1993; Felix *et al.*, 1999; Rao *et al.*, 1997). Evidence is now emerging for the involvement of plant subtilisin-like protease (Ser-PIs) in programmed cell death (PCD) (Vartapetian *et al.*, 2011), whereas *Pin2* was shown more than three decades ago to be a strong inhibitor of subtilisin (Plunkett *et al.*, 1982). The Kunitz trypsin Ser-PI encoded by AT1G73260, which is induced in response to SA, H₂O₂ and wounding treatments, has been shown to act as an antagonist of PCD induced by phytopathogens and fumonisins B1 (Li *et al.*, 2008). Interestingly, its closest homologue is AT1G17860 and, in this study, we showed that SGN-U312622 (AT1G17860) is part, together with *Pin2*, of the conserved gene set up-regulated in Rep₁₃₀-expressing plants. It will be interesting to evaluate whether PIN2 and the Ser-PI encoded by SGN-U312622 can act as inhibitors of aspartic-specific protein fragmentation

sustained by phytaspase and/or saspase, and whether they participate in the fine tuning of PCD-related phenomena.

Why is Rep₁₃₀ perceived as a stress? First, as Rep₁₃₀ is a subdomain of Rep, it may not be properly folded, and misfolded proteins can represent a stress signal. However, the observed functionality of plant-expressed Rep₁₃₀ tends to rule out this hypothesis, although we cannot rule out the possibility that a small fraction may be misfolded, thus acting as a stress trigger. Second, as the Rep₁₃₀ domain contains the conserved signatures of viral and plasmid rolling-circle replication proteins (Ilyna and Koonin, 1992), this catalytic domain could be recognized as a potential viral-associated molecular pattern, triggering defence-related responses. Third, TYLCSV and TGMV Reps have been shown to interact with SCE1 (Castillo *et al.*, 2004), and the TGMV Rep domain responsible for SCE1 binding was mapped in the Rep N-terminal domain (Sánchez-Durán *et al.*, 2011). Transient expression of TGMV Rep results in the alteration of the sumoylation pattern of a few specific plant proteins (Sánchez-Durán *et al.*, 2011); however, we did not identify this alteration in Rep₁₃₀-expressing plants (Fig. S5 see Supporting Information). Unfortunately, antibodies raised against *A. thaliana* SCE1 did not recognize tomato SCE1, thus precluding an analysis of the ability of tomato-expressed Rep₁₃₀ to bind it (data not shown). As SUMO conjugates accumulate rapidly in response to various adverse environmental conditions (Humberto Castro *et al.*, 2012; Kurepa *et al.*, 2003), it will be of interest from both a biotechnological and basic scientific point of view to analyse the behaviour of Rep₁₃₀-expressing plants under different growth and stress conditions. In addition, as the overexpression of Ser-PIs is known to confer resistance to some classes of insects, the outcome of the interaction between Rep₁₃₀-expressing plants and *Bemisia tabaci*, the vector of TYLCSV, deserves further focus.

In conclusion, we have shown that the ectopic expression of the TYLCSV Rep multifunctional catalytic domain confers high resistance to the homologous virus in tomato; however, it induces transcriptional changes resembling stress-related responses which partially overlap with those observed during TYLCSV and CaLCuV infections. A deeper transcriptional analysis using a different technology should hopefully lead to the discovery of additional host genes modulated by Rep₁₃₀. However, LongSAGE analysis has highlighted the capacity of the Rep N-terminal domain to modulate the mRNA steady-state level of host genes. It remains to be established whether this modulation plays a functional role in a productive viral infection.

EXPERIMENTAL PROCEDURES

Transgenic plants

Solanum lycopersicum 'MoneyMaker' was transformed with the recombinant *Agrobacterium tumefaciens* strain EHA105 harbouring the plasmid

pTOM130 (Lucioli *et al.*, 2003), as described in Brunetti *et al.* (1997). Western and Southern blot analyses on transgenic plants were carried out as described in Brunetti *et al.* (1997), except for protein extraction, which was performed by boiling 12 mg of liquid nitrogen-ground leaf tissue in 400 μ L Laemmli buffer for 5 min, followed by 5 min of centrifugation at 14 000 *g*.

TYLCSV infection and resistance assays

Plants were agroinoculated with *A. tumefaciens* strain C58C1 carrying a TYLCSV infectious clone or pBIN19 empty vector. Infection was monitored by dot blotting on nylon membrane DNA extracted according to Edwards *et al.* (1991); membranes were hybridized to digoxigenin- or 32 P-labelled C1-derived transcripts.

In vitro Rep₁₃₀ activity assay

For protein extraction, 12 mg of leaf tissue were ground in liquid nitrogen, resuspended in 200 μ L of extraction buffer [400 mM NaCl, 30 mM potassium phosphate buffer, pH 7.5, 10 mM β -mercaptoethanol, 1 $\times$ Complete™ Protease Inhibitor Cocktail (Roche Applied Science, Indianapolis, IN, USA)] and centrifuged at 16 000 *g* for 10 min at 4 °C. One microlitre of supernatant was incubated in a final volume of 10 μ L with 0.1 or 1 pmol of oligonucleotide gem12 (TAATATTACCGG), gem12-3 (TAATATTACCGGATG) or gem3-12 (GTATAATATTACCGG), or with 1 or 100 pmol of pJIT60 (TGGAGAGGACAGCCCAAG), for 30 min at 37 °C in cleavage buffer (Kittmann *et al.*, 2009) supplemented with 1 $\times$ Complete™ Protease Inhibitor Cocktail. Reactions were stopped by adding one volume of 2 $\times$ Laemmli buffer, boiled for 5 min and analysed by 15% SDS-PAGE and Western blotting.

Transcriptional profiling

Three independent sets of plants were grown in a glasshouse with a photoperiod of 16 h of light and 8 h of darkness. Each set consisted of five wild-type and six Rep130 transgenic plants (three from T2-406-27 and three from T2-410-11), randomly arranged. The first two expanded leaves below the apex were collected from 50-day-old wild-type and Rep130 transgenic plants (this plant age corresponds to a standard 4 weeks post-TYLCSV inoculation) and pooled, thus obtaining two samples (wild-type and Rep130) for each set of plants. Total RNAs were extracted from 2 g of tissue using Concert Plant RNA Reagent (Invitrogen, Carlsbad, CA, USA); RNAs from the three independent sets of plants were pooled from wild-type and from Rep130 samples. Twenty-five micrograms of total RNA from each sample were used to construct Long SAGE libraries, employing an I-SAGE Long Kit (Invitrogen). Sequencing was performed by a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using an ABI 3730 DNA Analyser (Biogen srl, Rome, Italy). Seventeen-nucleotide tag sequences were extracted from concatemer sequences using SAGEhaft-tagcalling software (Beissbarth *et al.*, 2004). The seventeenth nucleotide of each tag was not considered in further analyses, and only tags in which each nucleotide had a QV of no less than 20, as evaluated by basecaller KB™ software (<http://www.appliedbiosystems.com/>), were accepted; QV $\geq$ 20 predicts an error rate of $\leq$ 1%. Only tags with $P < 0.01$ (chi-squared 2 $\times$

2 test) and fold $\geq$ |2| were considered to be differentially expressed between the two libraries. Tags were associated with the corresponding SGN unigenes if their sequences were correctly positioned 3' adjacent to the last *M*/allI site (CATG) upstream to the polyA or to one of the polyAs for those unigenes with multiple terminations of RNA transcription, as highlighted by an inspection of expressed sequence tag (EST) sequences.

sqRT-PCR

sqRT-PCR was performed on RNA used to construct LongSAGE libraries, RNA from TYLCSV-infected or empty pBIN19-agroinoculated tomato plants, and RNA from transgenic tomato plants (M. Tavazza, unpublished data) for a synthetic Rep₁₃₀ transgene (*Rep130syn*) (Lucioli *et al.*, 2008). The reverse transcriptase reaction was primed with oligo(dT)₂₀ on 2.5 μ g of DNaseI-treated RNA using Superscript III (Invitrogen); for each sample, control reactions without reverse transcriptase were carried out in parallel. For sqRT-PCR, 1.5 μ L of a cDNA dilution (1:20) was amplified in 25 μ L PCR using Platinum Taq DNA Polymerase (Invitrogen) and specific oligonucleotides (Table S6, see Supporting Information). The reaction conditions were as follows: 2 min at 94 °C hot start, followed by a variable number of amplification cycles (94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s), depending on the abundance of the transcripts.

Bioinformatic analysis

Tag to gene assignment was initially performed by BLAST analysis using SGN tomato unigene Build 1 (<http://solgenomics.net/tools/blast/index.pl>) and NCBI (<http://blast.ncbi.nlm.nih.gov/>) as reference datasets, and then refined using the recently available tomato genomic sequence (Tomato WGS Chromosomes; SL2.40). GO term enrichment analysis of differentially expressed genes was performed using the Plant MetGenMap database (http://bioinfo.bti.cornell.edu/cgi-bin/MetGenMAP/GO_analysis.cgi). A GO category was considered to be enriched only when all three statistical tests in use at Plant MetGenMap (Bonferroni, FDR and simulation) gave a corrected $P < 0.01$. BiMax clustering analysis was conducted on manually curated high-quality microarray expression data of *S. lycopersicum* and *A. thaliana* leaf tissues available at the Genevestigator web-based interface (<https://www.genevestigator.com/gv/plant.jsp>) (Hruz *et al.*, 2008). Outputs from clustering analysis were only considered when at least five co-regulated genes were retrieved in two experimental conditions, with each gene in each condition having an FDR $P < 0.05$.

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REFERENCES

- Angel Torres, M. (2010) ROS in biotic interactions. *Physiol. Plant.* **138**, 414–429.
- Arguello-Astorga, G., Herrera-Estrella, L. and Rivera-Bustamante, R. (1994) Experimental and theoretical definition of geminivirus origin of replication. *Plant Mol. Biol.* **26**, 553–556.

- Arguello-Astorga, G.R. and Ruiz-Medrano, R. (2001) An iteron-related domain is associated to motif 1 in the replication proteins of geminiviruses: identification of potential interacting amino acid-base pairs by a comparative approach. *Arch. Virol.* **146**, 1465–1485.
- Ascencio-Ibanez, J.T., Sozzani, R., Lee, T.J., Chu, T.M., Wolfinger, R.D., Cella, R. and Hanley-Bowdoin, L. (2008) Global analysis of Arabidopsis gene expression uncovers a complex array of changes impacting pathogen response and cell cycle during geminivirus infection. *Plant Physiol.* **148**, 436–454.
- Bagewadi, B., Chen, S.J., Lal, S.K., Choudhury, N.R. and Mukherjee, S.K. (2004) PCNA interacts with *Indian mung bean yellow mosaic virus* Rep and downregulates Rep activity. *J. Virol.* **78**, 11 890–11 903.
- Beissbarth, T., Hyde, L., Smyth, G.K., Job, C., Boon, W.-M., Tan, S.-S., Scott, H.S. and Speed, T.P. (2004) Statistical modeling of sequencing errors in SAGE libraries. *Bioinformatics*, **20**, 31–39.
- Brunetti, A., Tavazza, M., Noris, E., Tavazza, R., Caciagli, P., Ancora, G., Crespi, S. and Accotto, G.P. (1997) High expression of truncated viral Rep protein confers resistance to *Tomato yellow leaf curl virus* in transgenic tomato plants. *Mol. Plant-Microbe Interact.* **10**, 571–579.
- Castillo, A.G., Colinet, D., Deret, S., Kashoggi, A. and Bejarano, E.R. (2003) Dual interaction of plant PCNA with geminivirus replication accessory protein (Rep) and viral replication protein (Rep). *Virology*, **312**, 381–394.
- Castillo, A.G., Kong, L.J., Hanley-Bowdoin, L. and Bejarano, E.R. (2004) Interaction between a geminivirus replication protein and the plant sumoylation system. *J. Virol.* **78**, 2758–2769.
- Chen, Z.X., Ricigliano, J.W. and Klessig, D.F. (1993) Purification and characterization of a soluble salicylic acid-binding protein from tobacco. *Proc. Natl. Acad. Sci. USA*, **90**, 9533–9537.
- Choudhury, N.R., Malik, P.S., Singh, D.K., Islam, M.N., Kaliappan, K. and Mukherjee, S.K. (2006) The oligomeric Rep protein of *Mungbean yellow mosaic India virus* (MYMIV) is a likely replicative helicase. *Nucleic Acids Res.* **34**, 6362–6377.
- Clerot, D. and Bernardi, F. (2006) DNA helicase activity is associated with the replication initiator protein Rep of tomato yellow leaf curl geminivirus. *J. Virol.* **80**, 11 322–11 330.
- Desbiez, C., David, C., Mettouchi, A., Laufs, J. and Gronenborn, B. (1995) Rep protein of tomato yellow leaf curl geminivirus has an ATPase activity required for viral-DNA replication. *Proc. Natl. Acad. Sci. USA*, **92**, 5640–5644.
- Eagle, P.A., Orozco, B.M. and Hanley-Bowdoin, L. (1994) A DNA-sequence required for geminivirus replication also mediates transcriptional regulation. *Plant Cell*, **6**, 1157–1170.
- Edwards, K., Johnstone, C. and Thompson, C. (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Res.* **19**, 1349.
- Egelkrout, E.M., Robertson, D. and Hanley-Bowdoin, L. (2001) Proliferating cell nuclear antigen transcription is repressed through an E2F consensus element and activated by geminivirus infection in mature leaves. *Plant Cell*, **13**, 1437–1452.
- Egelkrout, E.M., Mariconti, L., Settlege, S.B., Cella, R., Robertson, D. and Hanley-Bowdoin, L. (2002) Two E2F elements regulate the proliferating cell nuclear antigen promoter differently during leaf development. *Plant Cell*, **14**, 3225–3236.
- Elmer, J.S., Brand, L., Sunter, G., Gardiner, W.E., Bisaro, D.M. and Rogers, S.G. (1988) Genetic analysis of the *Tomato golden mosaic virus* II. The product of the AL1 coding sequence is required for replication. *Nucleic Acids Res.* **16**, 7043–7060.
- Fauquet, C.M., Briddon, R.W., Brown, J.K., Moriones, E., Stanley, J., Zerbini, M. and Zhou, X. (2008) Geminivirus strain demarcation and nomenclature. *Arch. Virol.* **153**, 783–821.
- Felix, G., Duran, J.D., Volko, S. and Boller, T. (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J.* **18**, 265–276.
- Fontes, E.P.B., Eagle, P.A., Sipe, P.S., Luckow, V.A. and Hanley-Bowdoin, L. (1994) Interaction between a geminivirus replication protein and origin DNA is essential for viral replication. *J. Biol. Chem.* **269**, 8459–8465.
- Graham, J.S., Hall, G., Pearce, G. and Ryan, C.A. (1986) Regulation of synthesis of proteinase inhibitor-I and inhibitor-II messenger RNAs in leaves of wounded tomato plants. *Planta*, **169**, 399–405.
- Green, T.R. and Ryan, C.A. (1972) Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. *Science*, **175**, 776–777.
- Gutzat, R. (2009) Functional characterization of the Arabidopsis retinoblastoma related protein. PhD thesis. DISS. ETH Nr. 18446. Available at: <http://e-collection.library.ethz.ch/eserv/eth:1005/eth-1005-02.pdf?pid=eth:1005&dsID=eth-1005-02.pdf> [accessed on March 10, 2013].
- Hanley-Bowdoin, L., Settlege, S.B. and Robertson, D. (2004) Reprogramming plant gene expression: a prerequisite to geminivirus DNA replication. *Mol. Plant Pathol.* **5**, 149–156.
- Hemler, M.E. (2008) Targeting of tetraspanin proteins: potential benefits and strategies. *Nat. Rev. Drug Disc.* **7**, 747–758.
- Herde, O., Fuss, H., Penacortes, H. and Fisahn, J. (1995) Proteinase-inhibitor-II gene-expression induced by electrical-stimulation and control of photosynthetic activity in tomato plants. *Plant Cell Physiol.* **36**, 737–742.
- Hruz, T., Laule, O., Szabo, G., Wessendorf, F., Bleuler, S., Oertle, L., Widmayer, P., Gruissem, W. and Zimmermann, P. (2008) Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. *Adv. Bioinformatics* **2008**, 420747.
- Humberto Castro, P., Tavares, R.M., Bejarano, E.R. and Azevedo, H. (2012) SUMO, a heavyweight player in plant abiotic stress responses. *Cell. Mol. Life Sci.* **69**, 3269–3283.
- Ilyina, T.V. and Koonin, E.V. (1992) Conserved sequence motifs in the initiator proteins for rolling circle DNA replication encoded by diverse replicons from eubacteria, eucaryotes and archaeobacteria. *Nucleic Acids Res.* **20**, 3279–3285.
- Jeske, H. (2009) Geminiviruses. *Curr. Top. Microbiol. Immunol.* **331**, 185–226.
- Joung, J.-G., Corbett, A.M., Fellman, S.M., Tieman, D.M., Klee, H.J., Giovannoni, J.J. and Fei, Z. (2009) Plant MetGenMAP: an integrative analysis system for plant systems biology. *Plant Physiol.* **151**, 1758–1768.
- Kemmerling, B., Schwedt, A., Rodriguez, P., Mazzotta, S., Frank, M., Abu Qamar, S., Mengiste, T., Betsuyaku, S., Parker, J.E., Muessig, C., Thomma, B.P.H.J., Albrecht, C., de Vries, S.C., Hirt, H. and Nuernberger, T. (2007) The BR1-associated kinase 1, BAK1, has a brassinolide-independent role in plant cell-death control. *Curr. Biol.* **17**, 1116–1122.
- Kittelmann, K., Rau, P., Gronenborn, B. and Jeske, H. (2009) Plant geminivirus Rep protein induces rereplication in fission yeast. *J. Virol.* **83**, 6769–6778.
- Kong, L.J., Orozco, B.M., Roe, J.L., Nagar, S., Ou, S., Feiler, H.S., Durfee, T., Miller, A.B., Gruissem, W., Robertson, D. and Hanley-Bowdoin, L. (2000) A geminivirus replication protein interacts with the retinoblastoma protein through a novel domain to determine symptoms and tissue specificity of infection in plants. *EMBO J.* **19**, 3485–3495.
- Kurepa, J., Walker, J.M., Smalle, J., Gosink, M.M., Davis, S.J., Durham, T.L., Sung, D.Y. and Vierstra, R.D. (2003) The small ubiquitin-like modifier (SUMO) protein modification system in Arabidopsis—accumulation of SUMO1 and -2 conjugates is increased by stress. *J. Biol. Chem.* **278**, 6862–6872.
- Lai, J., Chen, H., Teng, K., Zhao, Q., Zhang, Z., Li, Y., Liang, L., Xia, R., Wu, Y., Guo, H. and Xie, Q. (2009) RKP, a RING finger E3 ligase induced by BSCTV C4 protein, affects geminivirus infection by regulation of the plant cell cycle. *Plant J.* **57**, 905–917.
- Laufs, J., Traut, W., Heyraud, F., Matzeit, V., Rogers, S.G., Schell, J. and Gronenborn, B. (1995) *In vitro* cleavage and joining at the viral origin of replication by the replication initiator protein of *Tomato yellow leaf curl virus*. *Proc. Natl. Acad. Sci. USA*, **92**, 3879–3883.
- Lee, M.-H. and Sano, H. (2007a) Attenuation of the hypersensitive response by an ATPase associated with various cellular activities (AAA) protein through suppression of a small GTPase, ADP ribosylation factor, in tobacco plants. *Plant J.* **51**, 127–139.
- Lee, M.-H. and Sano, H. (2007b) Suppression of salicylic acid signaling pathways by an ATPase associated with various cellular activities (AAA) protein in tobacco plants. *Plant Biotechnol.* **24**, 209–215.
- Li, J., Brader, G. and Palva, E.T. (2008) Kunitz trypsin inhibitor: an antagonist of cell death triggered by phytopathogens and fumonisin B1 in Arabidopsis. *Mol. Plant* **1**, 482–495.
- Lozano-Duran, R., Rosas-Diaz, T., Luna, A.P. and Bejarano, E.R. (2011) Identification of host genes involved in geminivirus infection using a reverse genetics approach. *PLoS ONE*, **6**, e22383.
- Lozano-Duran, R., Garcia, I., Huguet, S., Balzergue, S., Romero, L.C. and Bejarano, E.R. (2012) Geminivirus C2 protein represses genes involved in sulphur assimilation and this effect can be counteracted by jasmonate treatment. *Eur. J. Plant Pathol.* **134**, 49–59.
- Lucioli, A., Noris, E., Brunetti, A., Tavazza, R., Ruzza, V., Castillo, A.G., Bejarano, E.R., Accotto, G.P. and Tavazza, M. (2003) *Tomato yellow leaf curl Sardinia virus* Rep-derived resistance to homologous and heterologous geminiviruses occurs by different mechanisms and is overcome if virus-mediated transgene silencing is activated. *J. Virol.* **77**, 6785–6798.
- Lucioli, A., Sallustio, D.E., Barboni, D., Berardi, A., Papacchioli, V., Tavazza, R. and Tavazza, M. (2008) A cautionary note on pathogen-derived sequences. *Nat. Biotechnol.* **26**, 617–619.

- Malik, P.S., Kumar, V., Bagewadi, B. and Mukherjee, S.K. (2005) Interaction between coat protein and replication initiation protein of *Mung bean yellow mosaic India virus* might lead to control of viral DNA replication. *Virology*, **337**, 273–283.
- Moriones, E. and Navas-Castillo, J. (2000) *Tomato yellow leaf curl virus*, an emerging virus complex causing epidemics worldwide. *Virus Res.* **71**, 123–134.
- Moura, D.S., Bergey, D.R. and Ryan, C.A. (2001) Characterization and localization of a wound-inducible type I serine-carboxypeptidase from leaves of tomato plants (*Lycopersicon esculentum* Mill.). *Planta*, **212**, 222–230.
- Nagar, S., Pedersen, T.J., Carrick, K.M., Hanley-Bowdoin, L. and Robertson, D. (1995) A geminivirus induces expression of a host DNA-synthesis protein in terminally differentiated plant-cells. *Plant Cell*, **7**, 705–719.
- Noirot, P., Bargonetti, J. and Novick, R.P. (1990) Initiation of rolling-circle replication in Pt181 plasmid-initiator protein enhances cruciform extrusion at the origin. *Proc. Natl. Acad. Sci. USA*, **87**, 8560–8564.
- Orozco, B.M., Kong, L.J., Batts, L.A., Elledge, S. and Hanley-Bowdoin, L. (2000) The multifunctional character of a geminivirus replication protein is reflected by its complex oligomerization properties. *J. Biol. Chem.* **275**, 6114–6122.
- Orozco-Cárdenas, M.L., Narváez-Vásquez, J. and Ryan, C.A. (2001) Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. *Plant Cell*, **13**, 179–191.
- Pautot, V., Holzer, F.M. and Walling, L.L. (1991) Differential expression of tomato proteinase inhibitor-I and inhibitor-II genes during bacterial pathogen invasion and wounding. *Mol. Plant-Microbe Interact.* **4**, 284–292.
- Pautot, V., Holzer, F.M., Reisch, B. and Walling, L.L. (1993) Leucine aminopeptidase: an inducible component of the defense response in *Lycopersicon esculentum* (tomato). *Proc. Natl. Acad. Sci. USA*, **90**, 9906–9910.
- Plunkett, G., Senear, D.F., Zuroske, G. and Ryan, C.A. (1982) Proteinase inhibitor-I and inhibitor-II from leaves of wounded tomato plants—purification and properties. *Arch. Biochem. Biophys.* **213**, 463–472.
- Preiss, W. and Jeske, H. (2003) Multitasking in replication is common among geminiviruses. *J. Virol.* **77**, 2972–2980.
- Prins, M., Laimer, M., Noris, E., Schubert, J., Wassenegeger, M. and Tepfer, M. (2008) Strategies for antiviral resistance in transgenic plants. *Mol. Plant Pathol.* **9**, 73–83.
- Rajan, V.B.V. and D'Silva, P. (2009) *Arabidopsis thaliana* J-class heat shock proteins: cellular stress sensors. *Funct. Integr. Genomics*, **9**, 433–446.
- Rama Devi, S., Chen, X., Oliver, D.J. and Xiang, C. (2006) A novel high-throughput genetic screen for stress-responsive mutants of *Arabidopsis thaliana* reveals new loci involving stress responses. *Plant J.* **47**, 652–663.
- Rao, M.V., Paliyath, C., Ormrod, D.P., Murr, D.P. and Watkins, C.B. (1997) Influence of salicylic acid on H₂O₂ production, oxidative stress, and H₂O₂-metabolizing enzymes—salicylic acid-mediated oxidative damage requires H₂O₂. *Plant Physiol.* **115**, 137–149.
- Rodrigo, G., Carrera, J., Ruiz-Ferrer, V., del Toro, F.J., Llave, C., Voinnet, O. and Elena, S.F. (2012) A meta-analysis reveals the commonalities and differences in *Arabidopsis thaliana* response to different viral pathogens. *PLoS ONE*, **7**, e40526.
- Roux, D., Vian, A., Girard, S., Bonnet, P., Paladian, F., Davies, E. and Ledoigt, G. (2006) Electromagnetic fields (900 MHz) evoke consistent molecular responses in tomato plants. *Physiol. Plant.* **128**, 283–288.
- Sánchez-Durán, M.A., Dallas, M.B., Ascencio-Ibanez, J.T., Reyes, M.I., Arroyo-Mateos, M., Ruiz-Albert, J., Hanley-Bowdoin, L. and Bejarano, E.R. (2011) Interaction between geminivirus replication protein and the SUMO-conjugating enzyme is required for viral infection. *J. Virol.* **85**, 9789–9800.
- Saracco, S.A., Miller, M.J., Kurepa, J. and Vierstra, R.D. (2007) Genetic analysis of SUMOylation in *Arabidopsis*: conjugation of SUMO1 and SUMO2 to nuclear proteins is essential. *Plant Physiol.* **145**, 119–134.
- Sardo, L., Lucioli, A., Tavazza, M., Masenga, V., Tavazza, R., Accotto, G.P. and Noris, E. (2011) An RGG sequence in the replication-associated protein (Rep) of *Tomato yellow leaf curl Sardinia virus* is involved in transcriptional repression and severely impacts resistance in Rep-expressing plants. *J. Gen. Virol.* **92**, 204–209.
- Saunders, K., Lucy, A. and Stanley, J. (1991) DNA forms of the geminivirus *African cassava mosaic virus* consistent with a rolling circle mechanism of replication. *Nucleic Acids Res.* **19**, 2325–2330.
- Selth, L.A., Dogra, S.C., Rasheed, M.S., Healy, H., Randles, J.W. and Rezaian, M.A. (2005) A NAC domain protein interacts with *Tomato leaf curl virus* replication accessory protein and enhances viral replication. *Plant Cell*, **17**, 311–325.
- Settlage, S.B., Miller, B. and Hanley-Bowdoin, L. (1996) Interactions between geminivirus replication proteins. *J. Virol.* **70**, 6790–6795.
- Settlage, S.B., Miller, A.B., Gruissem, W. and Hanley-Bowdoin, L. (2001) Dual interaction of a geminivirus replication accessory factor with a viral replication protein and a plant cell cycle regulator. *Virology*, **279**, 570–576.
- Shen, L., Kang, Y.G.G., Liu, L. and Yu, H. (2011) The J-domain protein J3 mediates the integration of flowering signals in *Arabidopsis*. *Plant Cell*, **23**, 499–514.
- Singh, D.K., Malik, P.S., Choudhury, N.R. and Mukherjee, S.K. (2008) MYMIV replication initiator protein (Rep): roles at the initiation and elongation steps of MYMIV DNA replication. *Virology*, **380**, 75–83.
- Stenger, D.C., Revington, G.N., Stevenson, M.C. and Bisaro, D.M. (1991) Replicational release of geminivirus genomes from tandemly repeated copies: evidence for rolling-circle replication of a plant viral DNA. *Proc. Natl. Acad. Sci. USA*, **88**, 8029–8033.
- Sun, J.-Q., Jiang, H.-L. and Li, C.-Y. (2011) Systemin/jasmonate-mediated systemic defense signaling in tomato. *Mol. Plant* **4**, 607–615.
- Thilmony, R., Underwood, W. and He, S.Y. (2006) Genome-wide transcriptional analysis of the *Arabidopsis thaliana* interaction with the plant pathogen *Pseudomonas syringae* pv. tomato DC3000 and the human pathogen *Escherichia coli* O157: H7. *Plant J.* **46**, 34–53.
- Trinks, D., Rajeswaran, R., Shivaprasad, P.V., Akbergenov, R., Oakeley, E.J., Veluthambi, K., Hohn, T. and Pooggin, M.A. (2005) Suppression of RNA silencing by a geminivirus nuclear protein, AC2, correlates with transactivation of host genes. *J. Virol.* **79**, 2517–2527.
- Turrà, D. and Lorito, M. (2011) Potato type I and II proteinase inhibitors: modulating plant physiology and host resistance. *Curr. Protein Pept. Sci.* **12**, 374–385.
- Vartapetian, A.B., Tuzhikov, A.I., Chichkova, N.V., Taliansky, M. and Wolpert, T.J. (2011) A plant alternative to animal caspases: subtilisin-like proteases. *Cell Death Differ.* **18**, 1289–1297.
- Wang, F., Vandepoele, K. and Van Lijsebettens, M. (2012) Tetraspanin genes in plants. *Plant Sci.* **190**, 9–15.
- Yang, Y., Qin, Y., Xie, C., Zhao, F., Zhao, J., Liu, D., Chen, S., Fuglsang, A.T., Palmgren, M.G., Schumaker, K.S., Deng, X.W. and Guo, Y. (2010) The *Arabidopsis* chaperone J3 regulates the plasma membrane H⁺-ATPase through interaction with the PKS5 kinase. *Plant Cell*, **22**, 1313–1332.
- Yun, B.-W., Feechan, A., Yin, M., Saidi, N.B.B., Le Bihan, T., Yu, M., Moore, J.W., Kang, J.-G., Kwon, E., Spoel, S.H., Pallas, J.A. and Loake, G.J. (2011) S-nitrosylation of NADPH oxidase regulates cell death in plant immunity. *Nature*, **478**, 264–268.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1 Western blot analyses of T1 transgenic lines and virus detection in *Tomato yellow leaf curl Sardinia virus* (TYLCSV)-agroinoculated T2 406-27 plants. (A) Western blot analysis of T1 progeny of lines 403, 406 and 410. WT, wild-type plants; C, positive control; + and – below the lane number indicate the presence or absence of the transgene, as evaluated by polymerase chain reaction (PCR). (B) Dot blot analysis of TYLCSV DNA levels in inoculated T2 406-27 plants. W1–W4, wild-type plants; 1, 7, 8, T2 406-27 segregant plants; 2–6, 9–12, T2 406-27 transgenic plants; H, noninoculated plant; +, total DNA from a TYLCSV-infected tomato plant.

Fig. S2 Leaf phenotype of transgenic T2 410-11 and T1 860 plants: +, transgenic plants; –, segregant plants.

Fig. S3 Analysis of *in vitro* cleavage activity of Rep₁₃₀ expressed in Rep130syn transgenic *Solanum lycopersicum* plants. Western

blot analysis of protein extracts from two Rep130syn transgenic *S. lycopersicum* T1 plants (860 and 867) incubated for 30 min at 37 °C with different concentrations of oligonucleotides, as reported above each lane.

Fig. S4 BiMax clustering of the *Arabidopsis thaliana* genes homologous to the core set of eight genes regulated in Rep130 and Rep130syn transgenic tomato plants. The selected bicluster (red box) represents three genes co-regulated in 15 conditions. *Arabidopsis thaliana* genes homologous to tomato unigenes are indicated above the box, tomato unigenes are reported in parentheses, perturbations are reported on the left and asterisks indicate protease inhibitor genes. AT2G31957, the *A. thaliana* homologue of SGN-U314484, is not represented on the *A. thaliana* ATH1 array.

Fig. S5 Analysis of sumoylation in Rep130 transgenic plants. Western blot analysis of protein extracts from Rep130 transgenic (T2 410-11) and wild-type *Solanum lycopersicum* plants; C, extracts from *Arabidopsis thaliana*. Antibody was anti-*A. thaliana*

Sumo 1 (abcam ab5316). Growth conditions: light/dark 16 h/8 h; 25 °C/16 °C.

Table S1 Association of Long Serial Analysis of Gene Expression (LongSAGE) tags to chromosomes, predicted cDNAs and Unigene sequences of *Solanum lycopersicum*.

Table S2 Gene ontology (GO) functional categories enriched in up-regulated genes of Rep130 transgenic tomato plants.

Table S3 *Arabidopsis thaliana* genes homologous to the 26 genes regulated in Rep130 transgenic tomato plants; co-regulated genes in the eight perturbation conditions highlighted by BiMax clustering analysis are reported.

Table S4 Gene ontology (GO) functional categories enriched in the co-regulated genes of Rep130syn and Rep130 transgenic tomato plants.

Table S5 Long Serial Analysis of Gene Expression (LongSAGE) tag frequencies of various wound response tomato genes.

Table S6 Primers used in semi-quantitative reverse transcription-polymerase chain reaction (sqRT-PCR).