Tobacco Mosaic Virus Infection Disproportionately Impacts Phloem Associated Translatomes in Arabidopsis thaliana and Nicotiana benthamiana

Tamara D. Collum[1,2], and James N. Culver[1,3]

[1]Institute for Bioscience and Biotechnology Research

[2]Department of Cell Biology and Molecular Genetics,

University of Maryland, College Park, MD 20742, USA

[3]Department of Plant Science and Landscape Architecture

University of Maryland, College Park, MD 20742, USA

Correspondence:

James N. Culver

5115 Plant Sciences Bldg

University of Maryland

College Park, MD 20854

jculver@umd.edu

Keywords Vascular Responses, Systemic Acquired Resistance, Plasmodesmata Gating, RNA Silencing, Basal Defense, Symptom Development

Abstract

In this study we use vascular specific promoters and a translating ribosome affinity purification strategy to identify phloem associated translatome responses to infection by tobacco mosaic virus (TMV) in systemic hosts *Arabidopsis thaliana* ecotype Shahdara and *Nicotiana benthamiana*. Results demonstrate that in both hosts the number of translatome gene alterations that occurred in response to infection is at least four fold higher in phloem specific translatomes than in non-phloem translatomes. This finding indicates that phloem functions as a key responsive tissue to TMV infection. In addition, host comparisons of translatome alterations reveal both similarities and differences in phloem responses to infection, representing both conserved virus induced phloem alterations involved in promoting infection and virus spread as well as host specific alterations that reflect differences in symptom responses. Combined these results suggest phloem tissues play a disproportion role in the mediation and control of host responses to virus infection.

1. Introduction

The ability of a plant virus to move systemically throughout its host is often essential to its biological success as well as a key factor in its ability to cause disease. Systemic movement generally requires the virus to gain access to the plant's vascular phloem. As the plant's main transport tissue, phloem functions in the systemic movement of a diverse set of molecules that include sugars, lipids, amino acids, nucleic acids, proteins, and phytohormones (Carella et al., 2016; Lucas et al., 2013; Turgeon and Wolf, 2009; Turnbull and Lopez-Cobollo, 2013). Many of these phloem mobile molecules are essential to the maintenance of plant physiology, development and the sensing and activation of stress and defense responses (Carella et al., 2016; Fu and Dong, 2013). Thus, the movement of molecules into and out of the phloem is highly regulated and represents a significant barrier to virus infection. Clearly, plant viruses have evolved to use phloem transport systems to establish infection in tissues distal to the original site of infection. Yet despite the importance of this transport tissue, relatively little is known about the molecular mechanisms of virus phloem loading or how the host phloem is altered during infection. Here we report on the translational gene alterations that occur within host vascular tissues in response to tobacco mosaic virus (TMV) infection.

The vascular phloem consists of sieve elements (SEs) and companion cells (CCs) surrounded by an array of support cells that include bundle sheath (BS) and phloem parenchyma (PP) (Knoblauch and Oparka, 2012; Turnbull and Lopez-Cobollo, 2013). At maturity CCs provide the genetic and metabolic capabilities to the conductive anucleate SEs via specialized branched plasmodesmata (PD) known as plasmodesmata pore units (PPUs) (van Bel, 1996). Virus access into the phloem requires cell-to-cell movement through PD connecting the surrounding support cells and CCs followed by movement into the SEs through the PPUs (Hipper et al., 2013). Passage

through each of these cell types and their connecting PD thus represent a potential barrier to systemic virus movement. As an example, resistance in soybean against cowpea chlorotic mottle virus has been linked to the blockage of virus movement from BS cells to phloem cells, suggesting the connection between these unique cell types represents a barrier to the systemic movement of this virus (Goodrick et al., 1991). Once in the SEs virus systemic movement occurs predominately via translocation, following the source to sink route of photosynthate (Gibbs, 1976; Hipper et al., 2013; Santa Cruz, 1999). Thus, modulating access to the vascular phloem appears to be a key determinant influencing systemic virus movement.

For viruses such as TMV, the type member of the genus *Tobamovirus*, cell-to-cell movement through PD requires both virus movement (MP) and replication proteins with PD transit occurring as a ribonucleoprotein (vRNP) complex, for review see (Carrington et al., 1996; Heinlein, 2015; Liu and Nelson, 2013). For systemic movement TMV also requires a functional capsid protein with virion assembly likely occurring within the SE (Callaway et al., 2001; Nelson and van Bel, 1998; Scholthof, 2005). TMV systemic movement is also impacted by host factors, including several components that bind the virus MP (Heinlein, 2015; Liu and Nelson, 2013; Ueki et al., 2010). For example, increased callose deposition within the vascular phloem has been associated with the overexpression of a cadmium-induced glycine-rich protein (cdiGRP), leading to the inhibition of *Tobamovirus* systemic movement (Ueki and Citovsky, 2002). Furthermore, reductions in PD associated callose deposition have been associated with a TMV MP - ankyrin repeat host protein interaction and can enhance virus movement (Ueki et al., 2010). Several lines of evidence also indicate that transcriptional reprogramming is essential for effective *Tobamovirus* systemic movement (Chen et al., 2013; Collum et al., 2016; Levy et al., 2013; Sasaki et al., 2009). Most recently, the targeted disruption of the CC expressed IAA26, an auxin indole, acidic acid

(Aux/IAA) transcriptional regulator was found to enhance TMV phloem loading and systemic movement (Collum et al., 2016). IAA26 disruption was associated with reductions in defense-associated responses including genes involved in the regulation of callose deposition. Combined these studies indicate that viruses such as TMV use an array of approaches to modulate PD and phloem characteristics in order to gain access to the plant's vasculature.

To date the specific impact of virus infections on the vascular phloem has been difficult to determine in part due to the technical difficulty of isolating such complex tissues. CCs and SEs form a pressurized system, and disruption of this system, as is done in many phloem sampling techniques can lead to the introduction of contaminates (Turgeon and Wolf, 2009). Other methods such as laser dissection require specialized equipment and can be costly to establish. In this study, we used translating ribosome affinity purification (TRAP) which utilizes His-FLAG-tagged ribosomal proteins expressed from tissue specific promoters (Mustroph et al., 2009; Reynoso et al., 2015). These tagged ribosomes are used to immuno-purify mRNA-ribosome complexes, referred to as the translatome. An advantage of this method is that it does not require invasive techniques or expensive equipment prior to mRNA harvesting. In addition, mRNAs associated with ribosomes are more likely to be in the process of translation and thus better represent the cellular condition than total cellular mRNA.

To investigate the effects of virus infection on the phloem translatome we expressed tagged ribosomes from two phloem specific promoters (pSUC2 and pSULTR2;2) as well as from the ubiquitously expressed cauliflower mosaic virus 35S promoter in two TMV systemic hosts, *Arabidopsis thaliana* ecotype Shahdara and *Nicotiana benthamiana*. Results from these studies demonstrate that leaf vascular phloem tissue is disproportionately regulated in response to TMV infection as compared to non-phloem tissues, confirming at the molecular level the importance of

modulating the vascular phloem barrier during virus infection. These studies also reveal both similarities and differences in host phloem responses to TMV infection. Genes showing similar phloem translatome alterations in both systemic hosts included those involved in the transport of the systemic acquired resistance (SAR) mobile signal and the production of phloem mobile siRNAs. These similarly altered phloem translatome genes likely represent conserved virus induced responses involved in modulating the phloem environment and/or promoting systemic virus movement. In contrast, host differential translatome responses such as those involved in the generation of reactive oxygen species likely reflect unique host responses that contribute to observed differences in symptoms, including the development of systemic necrosis in *N. benthamiana*. To our knowledge this study is the first profiling of the phloem translatome in response to virus infection.

2. Materials and methods

2.1 Translatome constructs and plant lines

Translatome constructs *p35S::HF-RPL18*, *pSUC2::HF-RPL18* and *pSULTR2;2::HF-RPL18* were kindly provided by Dr. J. Bailey-Serres, University of California, Riverside, CA, USA (Mustroph et al., 2009). For GUS expression constructs, upstream promoter sequences covering 2 kb upstream of the pSUC2 and pSULTR2;2 open reading frame were amplified from the provided translatome constructs using promoter-specific primers (Table S1). Cloned promoter fragments were moved into pBI101.1 (Clontech) directly upstream of the GUS reporter ORF via primer-generated Sal1 and BamH1 restriction sites to create *pSUC2::GUS* and *pSULTR2;2:GUS*. All constructs were introduced into the *Agrobacterium tumerfaciens* strain GV3101 (Holsters et al., 1978). *A. thaliana* ecotype Shahdara plants were transformed by the floral dip method (Clough and Bent, 1998). *N. benthamiana* plants were transformed by leaf disc transformation method

(Horsch et al., 1985). Transgene insertions were confirmed by qRT-PCR using primers specific for the His6-FLAG-RPL18 transcript (Table S1**).** Plants were maintained in growth chambers for a 12-h photoperiod, light 100 µmol s-1 m-2, at 24°C. No abnormalities in seedling growth or plant development were observed in any of the transgenic lines.

2.2 Virus inoculations and tissue immuno-prints

Four week old plants were used for all virus inoculations. Arabidopsis and *N. benthamiana* leaves were dusted with carborundum and mechanically inoculated with TMV (1 mg/ml) or mock infected with sterile water. Inoculated leaf tissue was collected after six days. For Arabidopsis the leaf tissue from 20 plants was combined for each biological replicate. For *N. benthamiana* leaf tissue from 9 plants was combined for each biological replicate. Two biological replicates were generated for each promoter construct. Biological replicates were grown in different months under the same growth chamber conditions (12-h photoperiod, light 100 µmol s-1 m-2 at 24°C).

To compare virus accumulations and spread inoculated leaves were placed onto nitrocellulose sheets and sandwiched between two sheets of filter paper leaving an imprint of the leaf. Tissue prints were washed in buffer (50 mM Tris pH 7.6, 150 mM NaCl) and blocked in 5% nonfat dry milk for 20 min at room temperature. Prints were then probed with rabbit anti-CP antiserum followed by incubation with alkaline phosphatase conjugated goat anti-rabbit antibodies (Sigma Chemical Company, St. Louis, MO USA). CP accumulation was visualized by the addition of 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium, as previously described (Knecht and Dimond, 1984).

2.3 Ribosomal associated mRNA purifications

Isolation of polysomes from *promoter::HF-RPL18* lines was done as previously described (Lin et al., 2014; Mustroph et al., 2009; Reynoso et al., 2015) with slight modifications. Frozen

tissue was homogenized in Polysome Extraction Buffer (PEB; 200 mM Tris-HCl, pH 9.0, 200 mM KCl, 25 mM ethylene glycol tetraacetic acid (EGTA) pH 8.0, 35 mM $MgCl_2$, 1% (v/v) octylphenyl-polyethylene glycol (Igepal CA-630), 1% (v/v) polyoxyethylene 10 tridecyl ether, 1% (v/v) sodium deoxycholate, 5 mM dithiothereitol (DTT), 1 mM PMSF, 50 ug/mL cycloheximide, 50 ug/mL chloramphenicol, 0.5 mg/mL heparin) using 10 mL PEB per 5 g of tissue. Homogenates were clarified by centrifugation at 16,000 *g* for 15 min and filtered with cheesecloth. For *N. benthamiana* samples, the supernatant was loaded onto an 8 mL 1.6 M sucrose cushion. Samples were ultracentrifuged at 170,000 g for 18 h at 4 ºC to pellet polysomes. 1 mL of PEB was used to resuspend the pellet.

IP of polysomes from *promoter::HF-RPL18* plants was done as previously described (Mustroph et al., 2009; Reynoso et al., 2015) with minor modifications. Anti-FLAG magnetic beads, 50 µL (Sigma Chemical Company, St. Louis, MO USA) were added to the resuspended pellet for *N. benthamiana* samples or directly to the clarified supernatant for Arabidopsis samples and incubated at 4 ºC overnight with gentle rocking. The beads were recovered using a magnet and washed four times for 5 min with 5 mL of wash buffer (200 mM Tris-HCl, pH 9.0, 200 mM KCl, 25 mM EGTA, 35 mM $MgCl_2$, 5 mM DTT, 50 µg/mL cycloheximide, 50 µg/mL chloramphenicol). The complexes were eluted by treatment of the magnetic beads with 100 uL of Elution Buffer (100 uL wash buffer, 10 uL of 5mg/mL $FLAG_3$ peptide - Sigma Chemical Company, St. Louis, MO USA, 0.5 uL of 2 U/mL RNAse OUT - Thermo Fisher Scientific Cleveland, Ohio, USA). RLT buffer plus 2-Mercaptoethanol from the Qiagen RNeasy kit (Qiagen, Valencia, CA USA) was added to the eluted complexes. A 0.5 X volume of 100% ethanol was then added and the sample transferred to Qiagen RNeasy columns. Washes and RNA elutions were performed according to the manufacturer's instructions. Isolated RNA was measured on a

NanoDrop 1000 and the quality of the RNA confirmed using a 2100 Bioanalyzer Eukaryotic Total RNA Nanochip (Agilent Technologies Palo Alto, CA USA).

2.4 RNA sequencing and analysis

Library preparation with Illumina TruSeq RNA sample preparation kit and sequencing with Illumina HiSeq1500 rapid run was done by the UM-IBBR Sequencing Core at University of Maryland College Park (http://ibbr.umd.edu/facilities/sequencing). Reads that passed Illumina's quality control filters were further processed. Reads were mapped to the *A. thaliana* Col-0 genome reference TAIR10 or the *N. benthamiana* draft genome v1.0.1 (Bombarely et al., 2012) using the CLC Genomics Workbench v 7.5.1 RNA-seq analysis tool and default parameters (Mismatch cost 2, Insertion cost 3, Deletion Cost 3, Length fraction 0.8, Similarity fraction 0.8, Max hits for a read 10 - CLC Bio, Aarhus, Denmark). Total reads aligned to genes were used in all subsequent analyses. Reads were similarly mapped to the TMV genome NC_001367 (Goelet et al., 1982) retrieved from NCBI (http://www.ncbi.nlm.nih.gov). The RNA-seq data were deposited in the Gene Expression Omnibus data repository (http://www.ncbi.nlm.nih.gov/geo/) under the accession numbers GSE97662 for *A. thaliana* samples and GSE97663 for *N. benthamiana* samples. Differentially expressed (DE) genes were identified using the CLC Genomics Workbench Baggerly's test on proportions as described in the CLC Genomics manual (http://resources.qiagenbioinformatics.com/manuals, Baggerly et al., 2003). This beta-binomial test compares the proportions of reads in one group against those of another group. Each sample is weighted based on the number of total reads to account for differences in total reads between samples. We then calculated a False Discovery Rate (FDR) correction for multiple-hypothesis testing (Benjamini and Hochberg, 1995). Results were filtered with a cut of value of fold change > 1.5, FDR corrected p-value < 0.05 for phloem enriched genes and fold change > 2 and >10, FDR

corrected p-value < 0.05 for TMV altered genes. Gene symbols, and gene model descriptions were retrieved from TAIR (Lamesch et al., 2012). The blastx tool within the CLC genomics workbench was used with default parameters to identify the best Arabidopsis protein match in the TAIR10_pep_20110103 protein database based on bit score for each *N. benthamiana* DE gene.

2.5 Gene ontology and heatmap generation

Gene ontology (GO) enrichment analysis of DE genes was performed using agriGO, an agriculturally focused web based GO analysis program (Du et al., 2010). The singular enrichment analysis tool was used to identify GO terms that were significantly enriched (FDR p-value <0.05) among TMV altered genes using the *A. thaliana* genome as the background. The analysis was done independently for both up and down regulated genes. Transcription factors were identified using the Arabidopsis gene regulatory information server (AGRIS), AtTFDB (Yilmaz et al., 2011). All heatmaps were generated in R using the heatmap.2 function in the gplots CRAN library (http://cran.r-project.org/web/packages/gplots/index.html). For GO enrichment heatmaps the FDR p-value was used. For gene expression heatmaps Log_2 (Fold Change) values of TMV infected compared to mock infected samples exported from the CLC genomics workbench were used.

2.6 qRT-PCR validation of select transcripts

RNA for quantitative RT-PCR (qRT-PCR) validation was isolated from captured polysomes from *pSUC2::HF-RPL18* plants as previously described for two additional biological replicates. For these studies 300 ng of isolated RNA was pretreated with RQ1 DNase (Promega, Madison, WI USA), followed by reverse transcription using SuperScript III First-Strand Synthesis System and random hexamer primers (Invitrogen by Life Technologies, Carlsbad, CA USA). SYBR green real-time qRT-PCR was performed in an ABI Prism 7100 (Applied Biosystems, Foster City, CA USA). Experiments were done in two biological replicates, with each containing

three technical replicates. 18S rRNA was chosen as an internal control for normalization. Primer sequences used for the amplification of all selected genes are provided in Table S1.

3. Results

3.1 Characterization of plant lines and translatome system

To investigate phloem alterations during virus infection, plasmids encoding ribosomal protein L18 (RPL18) tagged with a His6-FLAG dual-epitope under the control of the Arabidopsis phloem promoters pSUC2 and pSULTR2;2 as well as the more ubiquitously expressed CaMV 35S promoter were transformed into the systemic TMV hosts *A. thaliana* ecotype Shahdara and *N. benthamiana* (Dardick et al., 2000). qRT-PCR analysis confirmed the expression of *HF-RPL18* transcripts for each promoter line. Interestingly, promoter expression levels in Arabidopsis were similarly low while expression levels in *N. benthamiana* were considerably higher for the p35S and pSULTR2;2 constructs (Fig. 1A). Higher levels of transgene expression within *N. benthamiana* are not uncommon and are consistent with this plants selection as a protein expression system (Goodin et al., 2008).

All transgenic plant lines were phenotypically indistinguishable from non-transformed control plants (Fig. 1B). Previous studies have shown that the pSUC2 promoter drives expression predominately in shoot CCs while the pSULTR2;2 expresses in both shoot CCs and bundle sheath cells (Mustroph et al., 2009; Stadler and Sauer, 1996; Takahashi et al., 2000; Truernit and Sauer, 1995). The p35S promoter drives near constitutive expression in most cell types including vascular tissue (Benyon et al., 2013; Kay et al., 1987; Sunilkumar et al., 2002). Reporter lines confirming the tissue specificity of these promoters have been previously reported for Arabidopsis (Mustroph et al., 2009). In this study, GUS reporter lines were used to validate the vascular expression of the pSUC2 and pSULTR2;2 promoters in *N. benthamiana* (Fig. 1C). Arabidopsis and *N. benthamiana* plants were selected as both permit similar levels of TMV spread and accumulation in inoculated

leaf tissues as well as rapid systemic virus movement (Fig. 2A). However, TMV induces leaf curling and stunting in *A. thaliana* ecotype Shahdara and systemic necrosis in *N. benthamiana* (Fig. 2B & C). Thus, these hosts provide a system to investigate phloem associated alterations in two TMV host's that are similarly permissive to systemic virus spread but unique in their symptom responses.

For experiments, four-week-old T2 plants from two independent lines of each promoter construct were inoculated with TMV (1 mg/ml) or mock inoculated with sterile water. Inoculated leaf tissue was harvested 6 days post infection (dpi), a time point at which TMV infections displayed similar levels of spread and accumulation along with the first visual symptoms within the systemic leaves of *A. thaliana* and *N. benthamiana* (Fig. 2) (Dardick et al., 2000). Inoculated leaf tissue from 20 plants for Arabidopsis or nine plants for *N. benthamiana* was pooled for each biological replicate, for a total of two replicates per biological condition. Biological replicates were collected four months apart for Arabidopsis and one month apart for *N. benthamiana***.** To control for environmental and circadian effects, plants were maintained under identical growth conditions of a 12-h photoperiod at 24°C with inoculations and tissue collections done in the late afternoon each day.

3.2 Phloem associated translatome analysis

Approximately 29 to 60 million 50 bp single-end sequence reads were generated for each Arabidopsis biological condition and 94 to 116 million reads for each *N. benthamiana* biological condition (Table S2). Reads were mapped to the *A. thaliana* Col-0 genome reference TAIR10 or the *N. benthamiana* draft genome sequence v1.0.1 as well as the TMV genome (NCBI Reference Sequence NC_001367) using the CLC Genomics Workbench v 7.5.1 (Bombarely et al., 2012; Goelet et al., 1982; Lamesch et al., 2012; Mortazavi et al., 2008).

For Arabidopsis samples, 97 to 98% of reads from mock infected samples and 80 to 94% of reads from TMV infected samples mapped to the Arabidopsis genome (Table S2). For *N. benthamiana* samples, 79 to 90% of reads for mock infected samples and 69 to 91% of reads for TMV infected samples mapped to the *N. benthamiana* draft genome (Table S2). Up to 1.73% or 1.37% of reads from Arabidopsis or *N. benthamiana* infected samples, respectively, mapped to the TMV genome. This similarity in the reads mapping to the TMV genome is consistent with the similar levels of virus spread and accumulation observed at 6 dpi in both hosts (Fig. 2A). All mock infected samples for both hosts have less than 0.01% of reads mapping to the TMV genome and these reads map to the 5' TMV Ω sequence used as a transgene translational enhancer (Fig. S1). Pairwise comparisons showed that all biological replicates had a Pearson correlation between 0.93 and 0.99 (Fig. S2 & S3) indicating high reproducibility between biological replicates.

The presence of known phloem associated genes was used to confirm tissue specificity for pSUC2 and pSULTR2;2 translatomes. We defined phloem enriched genes as those genes that are significantly up regulated (Fold Change > 1.5, FDR P-Value < 0.05) in pSUC2 mock or pSULTR2;2 mock samples compared to p35S mock samples using the CLC genomics workbench test on proportions (Baggerly et al., 2003) (Dataset S1). Arabidopsis pSUC2 and pSULTR2;2 phloem enriched genes were compared to previously published shoot phloem translatome data. In summary, we found that 35/203 (17.24%) of identified Arabidopsis phloem enriched genes overlapped with previously reported Arabidopsis shoot phloem enriched genes (Mustroph et al., 2009). Conversely, none of the 46 Arabidopsis p35S translatome enriched genes overlapped with published shoot phloem translatome data (Dataset S1). Additionally, 85/717 (10.60%) of the previously identified Arabidopsis shoot phloem enriched genes were identified as phloem enriched in *N. benthamiana* in this study (Mustroph et al., 2009). These percent overlaps are consistent

with previous comparisons of other phloem studies that showed 8.90% and 21.80% overlap (Deeken et al., 2008; Mustroph et al., 2009; Zhao et al., 2005). The low percent of overlap between phloem studies can be explained by differences in sampling techniques and plant age, which ranged from 1 week to 10 week-old plants. However, combined these studies are consistent with the successful isolation of phloem associated translatome mRNA.

3.3 TMV's impact on host and tissue associated translatomes.

Translatome alterations in response to TMV infection were identified by comparison of mock and TMV inoculated biological replicates for each promoter construct. DE genes (fold changes >2 to 10 or >10, FDR P-Value < 0.05 for Arabidopsis and for *N. benthamiana*) were identified via the CLC genomics workbench test on proportions (Baggerly et al., 2003). Interestingly, in total we identified significantly more genes as altered in response to TMV infection in *N. benthamiana* (34,155) as compared to Arabidopsis (5,774). This host difference was even greater for highly altered genes with fold changes >10 with 10,501 genes from *N. benthamiana* and only 536 genes in Arabidopsis (Fig. 3). The larger genome size of *N. benthamiana* may account for a portion of this greater gene response. In addition, lower expression levels of the *HF-RPL18* transgenes in Arabidopsis may also have reduced the overall capture of ribosome-associated mRNAs in this host (Fig. 1A). However, this response variance also reflects symptom differences between the two hosts with *N. benthamiana* displaying systemic necrosis shortly after the 6 dpi translatome sampling time (Fig. 2C).

For additional analysis we separated out affected gene alterations into 1) phloem associated; gene alterations that occurred only in one or both of the two phloem (pSUC2 and pSULTR2;2) translatomes, 2) non-phloem associated; changes that occurred only in the p35S translatomes and 3) whole leaf tissues; alterations found in at least one of the phloem translatomes

and the p35S translatome. Comparison of phloem to non-phloem associated changes revealed that in both hosts the majority, >80% of observed gene alterations, occurred in phloem associated translatome (Fig. 3). In addition, compared with the whole leaf translatomes the number of genes that display a significant response to TMV were highest in the phloem associated translatomes for Arabidopsis at both the >2 to 10 and >10-fold cutoff levels as well as at the >10-fold cutoff for *N. benthamiana* (Fig. 3). These findings suggest that TMV's impact as well as host responses are greatest within the vascular tissues.

Comparison of genes altered in response to infection indicates that both Arabidopsis and *N. benthamiana* phloem translatomes are significantly altered but that both display unique gene regulation patterns. In Arabidopsis, 92% of the phloem associated genes altered >2 to 10-fold were down regulated, while only 24% of the altered non-phloem genes and 41% of the altered whole leaf genes were down regulated (Fig. 3A, Dataset S2). A similar pattern was seen for >10-fold altered genes with 86% of phloem associated genes down regulated, while only 22% of the altered non-phloem genes and 45% of the whole leaf genes were down regulated (Fig. 4C, Dataset S2). Thus, the phloem translatome of Arabidopsis primarily responds negatively to TMV infection while its corresponding non-phloem translatome displays a predominately positive gene regulation response. In contrast, the phloem and non-phloem translatomes of *N. benthamiana* display similar ratios of up and down regulated genes in all tissues with only 29% to 38% of TMV responsive genes down regulated in the >2 to 10-fold group and 29% to 40% of >10-fold altered genes down regulated (Fig. 4B & D, Dataset S3). Thus, the overall impact of TMV on tissue specific translatome regulation varies within these different hosts.

3.4 Similarly regulated host biological processes

To better compare host responses a BLAST analysis was used to group translatome identified *N. benthamiana* genes with their closest counterpart in Arabidopsis. For this analysis we focused on the most significantly affected genes, including only *N. benthamiana* genes with a >10-fold change. This resulted in 4,713 unique genes out of the original 10,501 TMV affected genes, as many *N. benthamiana* genes BLAST aligned to the same Arabidopsis gene (Dataset S4). TMV altered genes from both hosts were then analyzed for their inclusion in specific gene ontology (GO) biological processes using agriGO analysis tools (Du et al., 2010). This GO analysis was done independently for both up and down regulated genes. GO biological processes that were significantly over-represented in terms of having greater numbers of altered genes than would be expected by chance for the Arabidopsis genome were identified using the agriGO GO singular enrichment analysis tool (Du et al., 2010). The significance of these GO processes within phloem, whole leaf and non-phloem infected tissues for both hosts (FDR p-value < 0.05) were determined for both up and down regulated genes and compared (Fig. 5).

Within non-phloem tissues no over-represented biological processes were identified as shared by the two hosts. In whole tissues only two similarly regulated biological processes were shared between Arabidopsis and *N. benthamiana*. Both similar regulated GO processes, cation and ion transport, were over represented among up regulated genes in response to infection with the majority of the genes composing these processes involved in energy production and transfer (Fig. 5).

In both hosts the phloem associated tissue contained the highest number of shared over-represented GO biological processes among genes that were changed during infection, nine similar regulated and 34 differentially regulated (Fig. 5). Again, indicating that TMV induces significant alterations within phloem associated tissues regardless of host. For similarly regulated phloem

associated responses the two hosts share only one over-represented GO biological process among up regulated genes and eight over-represented GO biological processes among down regulated genes (Fig. 5A). The one shared over-represented GO process among up regulated phloem genes was lipid localization, which is of particular interest as this category contained genes involved in the mobility and maintenance of SAR and induced systemic resistance. Over-represented GO terms shared among phloem associated down regulated genes included those for stimulus response, metabolic processes and development and includes genes involved in an array of different functions including transcription and hormone signaling (Fig. 5A).

3.5 Differentially regulated host biological processes

Of the significantly over-represented biological processes shared between the two hosts most were differentially regulated (Fig. 5B). Of the 34 biological processes showing opposite responses none were found in the non-phloem tissues, four were identified in whole leaves, and 30 were associated with the phloem. The four oppositely regulated whole leaf processes were all up regulated in Arabidopsis and down regulated in *N. benthamiana*. These biological processes included genes involved in oxidation reduction and the electron transport chain. The up regulation of these GO processes in Arabidopsis may reflect this host's attempt to mitigate stress related processes associated with infection. In contrast, the down regulation of these processes in *N. benthamiana* likely reflects alterations that result from the development of necrosis. Of the 34 phloem associated biological processes all were up regulated in *N. benthamiana* and conversely down regulated in Arabidopsis phloem (Fig. 6B). This included GO processes involving responses to stimulus and stress, both categories that contain a number of defense associated genes including genes involved in RNA silencing. In addition, the majority of the over-represented GO processes in *N. benthamiana* phloem altered genes were also over-represented in *N. benthamiana* whole leaf

altered genes, a finding consistent with the higher number of genes and greater impact TMV has on the translatomes of this host (Fig. 6B). Combined these findings indicate that alterations within the vascular tissues dominate host selective responses to infection.

3.6 Identification of specific host pathways and gene families in response to TMV infection

Gene families and pathways known from the literature to be associated with host defense responses including transcription factors, lipid transfer genes, RNA silencing, callose synthases, reactive oxygen species (ROS) metabolic processes, and SAR were selected for further analysis (Fig. 6 to 9). We compared gene category lists collected from TAIR with our list of genes significantly altered during TMV infection identified using the CLC genomics workbench test on proportions (Baggerly et al., 2003). Analysis of these individual gene families and pathways uniquely outlines differences between the two TMV hosts with the majority of TMV altered genes being down regulated in the Arabidopsis pSUC2 phloem translatomes and up regulated in pSUC2, pSULTR2;2 and p35S in *N. benthamiana* translatomes (Fig. 6 to 9). The exception being the lipid transfer related genes, which primarily are up regulated in both hosts (Fig.7). These findings are consistent with the overall translatome regulation patterns observed for these hosts and reveal the identity of individual genes whose function may directly impact the TMV infection processes. Details regarding the importance and regulation of individual genes are discussed below.

3.7 Validation of RNA-Seq expression analysis

Twelve genes from each host that were associated with host defense responses were selected for further expression analysis using qRT-PCR. Two additional translatome biological replicates derived from different plant sets were collected from *pSUC2::HF-RPL18 A. thaliana* and *N. benthamiana* plants infected with TMV or mock infected as previously described. qRT-PCR results showed that that in both hosts all 12 genes displayed transcriptional alterations in response

to TMV infection that were similar in response to that observed in the RNA-Seq translatome studies (Table 1). Combined these findings indicate that observed translatome alterations are consistent within this study.

4. Discussion

Viruses must usurp normal phloem functions to facilitate their systemic spread from the initially site of infection to distal tissues (Hipper et al., 2013; Wang, 2015). In this study we investigated the extent to which TMV modulates the translatome of its host's vascular tissues. A unique attribute of this translatome approach is that it allows for the selection of tissue specific ribosome associated mRNAs (Mustroph et al., 2009; Reynoso et al., 2015). For our studies we utilized two phloem tissue specific promoters, pSUC2 and pSULTR2;2, and one non-tissue specific promoter, p35S, to drive expression of a tagged ribosomal protein RPL18, providing a snapshot into the pool of phloem and leaf specific mRNAs that are likely being translated and thus directly impacting cellular function. Results from this study reveal the preferential and significant impact TMV infection has on the vascular phloem in terms of the numbers and functions of affected genes.

For these studies phloem associated gene alterations were considered to be changes in ribosomal associated mRNA abundance that occurred only in the pSUC2 or pSULTR2;2 translatome profiles, while alterations that occurred specifically in the constitutively expressed 35S translatome were considered as non-phloem alterations. Gene alterations that overlapped between phloem associated translatomes and the p35S translatomes were considered as representing alterations that occurred in all leaf tissues and were listed as whole leaf changes. Surprisingly, in response to infection phloem associated translatome alterations were four fold higher than non-phloem alterations in both Arabidopsis and *N. benthamiana* (Fig. 3 & 4). In

addition, with the exception of the lower magnitude alterations in *N. benthamiana* (2 - 10-fold changes) phloem associated gene alterations in both hosts were either similar or significantly greater in terms of the total number of altered genes than observed in whole leaf tissues. Furthermore, higher numbers of TMV altered genes identified in phloem associated translatomes do not correlate with promoter expression levels as all three promoters display similar levels of expression in Arabidopsis while in *N. benthamiana* expression levels for the pSUC2 and pSULTR2;2 phloem promoters are significantly lower than the p35S promoter, yet these phloem promoters yield similar or greater numbers of altered genes in this host (Fig. 1A & 3). This disproportionate impact on phloem associated tissues is further reflected in the number of significantly represented biological GO processes identified as altered in response to infection with 86% identified as specific to the phloem (Fig. 5). Combined these findings strongly indicate that phloem and its associated tissues function as key contributors in host-virus responses and as such are also a target for virus directed reprogramming aimed at overcoming host responses and facilitating systemic infection.

Comparisons of host responses between Arabidopsis and *N. benthamiana* provided the means to identify both common and specific host responses. One striking difference between these two hosts are the differences between whole leaf and non-phloem associated responses to TMV (Fig. 3). In Arabidopsis, genes identified from the constitutive p35S derived translatomes make up ~13% of all altered genes, including those overlapping with other promoters, while in *N. benthamiana* p35S derived translatomes make up ~59% of all altered genes (Fig. 3). Some of this difference is likely related to the higher p35S expression levels observed in *N. benthamiana,* leading to the identification of greater numbers of altered genes in all tissues (Fig. 1A). However, other factors such as the more robust symptom response that includes the development of necrosis

in *N. benthamiana* may also contribute to this difference. A second difference involves the direction of gene regulation in the phloem associated translatomes with 91% of genes being down regulated in Arabidopsis while 70% are up regulated in *N. benthamiana* (Fig.4). This difference in the direction of gene regulation is another indication that within the phloem these two hosts have uniquely different responses to TMV. Again, host specific responses, especially the development of systemic necrosis in *N. benthamiana* may account this difference, however additional studies will be needed to clarify this response variation.

The examination of translatome alterations that are shared between the two hosts likely denote both common host defense and stress related changes that occur during infection as well as specific virus directed responses required to establish a successful infection. Interestingly, for whole leaf changes, those representing similar translatome changes found in all tissues, only two GO processes were identified as significantly over-represented. These processes involve genes for ion and cation transport that typically contribute to energy transduction, stress responses and the movement and redistribution of nutrients (Hedrich, 2012). Not unexpectedly, these genes were up regulated in both Arabidopsis and *N. benthamiana*, indicating a need in all tissues to increase nutrient availability, intracellular energy production and mediated stress demands imposed during virus infection.

Additional host comparisons of shared GO processes revealed the majority of over-represented GO categories were phloem associated, derived from the pSUC2 and pSULTR2;2 translatomes (Fig. 5). Of these only one GO term, lipid localization, was over-represented among up regulated genes from both hosts (Fig. 5). This category is of significant interest as it contains a number of genes involved in lipid transport including Early Arabidopsis Aluminum Induced 1 (EARLI1) (Fig. 7). EARLI1 has been shown to play an essential role in the propagation of the

SAR / ISR mobile signal (Cecchini et al., 2015; Isaacs et al., 2016). In addition, Defective in Induced Resistance 1 (DIR1) is also up regulated in the *N. benthamiana* phloem translatome and has been shown to function in the phloem mobilization of the SAR signal (Cecchini et al., 2015; Isaacs et al., 2016). Both EARLI1 and DIR1 are known to interact and likely form part of an SAR signaling complex (Cecchini et al., 2015; Yu et al., 2013). The fact that these genes are up regulated in phloem associated tissues during TMV infection indicates that systemic host defense responses are activated but that the virus is either capable of disrupting or outrunning this defense.

Within shared phloem associated biological processes were a number of specific gene categories and pathways with known associations to virus infection and host defense responses. One such gene category was transcription factors whose members contribute to the regulation of both plant growth and defense responses (Fig. 6). An analysis of all phloem altered TFs shows slightly more, 37 of 67, display opposite host responses to TMV infection with all 37 being down regulated in Arabidopsis and up regulated in *N. benthamiana*. . In addition, greater numbers of TMV altered *N. benthamiana* TFs were present in the p35S translatome than in the corresponding Arabidopsis translatome (Fig. 6). These differentially regulated TFs likely have a direct impact on the distinct responses within these two hosts.

Only three TFs and their associated alleles in *N. benthamiana* were up regulated in both hosts. These included MYB domain proteins MYB15 and MYB102 as well as a NAC domain containing protein, ANAC071 (Fig. 6). MYB15 and MYB102 are involved in response to salt stress and have also been implicated in response to wounding or defense responses to insect herbivores (De Vos et al., 2006; Denekamp and Smeekens, 2003; Dubos et al., 2010). ANAC071 expression is auxin regulated and found to promote cell division along artificially induced stem incisions as part of the wound healing and tissue reunion process (Asahina et al., 2011). The up

regulation of these TFs in both hosts and their association with stress, cell division and wounding suggests they are involved in cellular responses to TMV infection.

Of the TFs that were down regulated in both hosts a number were involved in hormone regulation. Changes to the expression of these TFs could influence the balance between plant growth and defense. For example, the DELLA subfamily member, RGA-LIKE1 (RGL1), was down regulated in both the pSUC2 and pSULTR2;2 phloem translatomes in both Arabidopsis and *N. benthamiana.* DELLA proteins act as repressors of GA signaling to control plant growth and development (Davière and Achard, 2013; Sun, 2011). DELLA proteins are also involved in cross-talk with multiple other plant hormone pathways including jasmonic acid, ethylene and auxin pathways (Davière and Achard, 2016; De Bruyne et al., 2014; Hou et al., 2013). Additionally, two related transcription factors aintegumenta (ANT) and aintegumenta-like6 (AIL6) were also down regulated in the phloem of both hosts. While these transcription factors are traditionally associated with auxin signaling and flower development they have also been linked to defense responses as *ant ail6* plants were shown to have elevated levels of salicylic acid and jasmonic acid and increased resistance to *Pseudomonas syringae* (Krizek, 2009; Krizek et al., 2016).

Another gene category of interest is callose synthases (CalS). Callose deposition at plasmodesmata has been demonstrated to play an important role in modulating the cell-to-cell movement of several plant viruses including TMV (Burch-Smith and Zambryski, 2016; Iglesias and Meins, 2000; Li et al., 2012). Eleven of the 20 identified CalS that display alterations in the phloem of both hosts were down regulated in Arabidopsis and up regulated in *N. benthamiana*. This pattern of opposite gene responses is consistent with the overall gene regulation differences observed between these two hosts (Fig.4 and 8A). However, six of the identified phloem altered CalS were negatively regulated in both hosts. The down regulation of these CalS genes in both

susceptible hosts suggests that their activity during virus infection may be disrupted. Three of these CalS genes, CalS1, CalS3 and CalS8 are known to be PD associated and can affect the gating of molecules from cell-to-cell (Cui and Lee, 2016; Vatén et al., 2011). Thus, it may be advantageous for virus movement if these CalS are reduced in expression.

RNA silencing associated genes represents another gene category of interest for its role in anti-viral defenses. Key RNA silencing genes that function in the creation of virus-derived small interfering RNAs (vsiRNAs) include dicer-like (DCL) endonucleases, host RNA dependent RNA polymerases (RDR), and argonaute ribonuclear proteins (AGO) (Qi et al., 2009 Zhang et al 2015). We found three dicer-like genes, DCL 1/2/4, were down regulated in Arabidopsis phloem but up regulated in *N. benthamiana*, suggesting the RNA silencing response may be uniquely affected in each host (Fig. 8B). DCL 4/2 have been identified as key contributors in the production of vsiRNAs although DCL3 has also been shown to play a minor role in antiviral defense (Deleris et al., 2006; Zhang et al., 2015). Unlike the other dicer-like genes, DCL3 was down regulated specifically in the phloem translatomes of both Arabidopsis and *N. benthamiana*. This is of interest as DCL3 processes the production of 24 nt sRNAs from endogenous genes and grafting experiments have demonstrated these 24 nt sRNAs are selectively phloem mobile (Deleris et al., 2006; Melnyk et al., 2011; Molnar et al., 2010). Thus, a reduced level of DCL3, specifically within the phloem of both hosts suggests that TMV infection disrupts the systemic mobility of these RNA signals.

AGOs function in the targeting of vsiRNAs to complementary viral transcripts via the RNA induced silencing complex (Carbonell and Carrington, 2015). The Arabidopsis genome encodes ten AGOs classified into 3 clades with varied and often overlapping functions in RNA virus defenses (Mallory and Vaucheret, 2010; Zhang et al., 2015). In our study we identified AGOs

1/2/6/7/10 as down regulated specifically in the phloem of Arabidopsis. In comparison, *N. benthamiana* alleles of AGOs 6/10 were similarly down regulated (Fig. 8B). AGO 10 belongs to clade 1 that primarily bind 21 nt siRNAs involved virus induced silencing while AGO 6 is a member of clade 2, which bind 24 nt sRNAs derived from endogenous gene elements (Czech and Hannon, 2011; Takeda et al., 2008). Down regulation of the 24 nt binding AGO 6 corresponds with the down regulation of DCL3 in both hosts and suggests that impairment of associated phloem mobile 24 nt sRNAs may be a key feature of TMV infection.

RDRs function in the amplification of siRNAs by producing additional dsRNAs for subsequent AGO cleavage and processing (Wang et al., 2010; Zhang et al., 2015). In Arabidopsis RDRs 1/2/6 have been demonstrated to function in viral defense responses with RDRs 1/6 specifically shown to be involved in the amplification TMV-Cg vsiRNAs (Garcia-Ruiz et al., 2010; Qi et al., 2009; Zhang et al., 2015). In our study, RDR 1/2/6 were significantly down regulated only in the pSUC2 phloem translatome of Arabidopsis. In *N. benthamiana* RDR 1 was up regulated in phloem and whole tissue translatomes while RDR 6 was up regulated only in the pSUC2 phloem translatome (Fig. 8B). From these findings it appears that Arabidopsis RNA silencing mechanisms are either not affected or targeted for reduced expression levels, specifically within the phloem. In contrast, the up regulation of several key silencing components in *N. benthamiana* suggests the mounting of a more robust defense response to TMV in this host. Within the *N. benthamiana* RDR 1 transcript an insertion conferring premature stop codons is linked to the enhanced susceptibility of this host to TMV and several additional RNA viruses (Yang et al., 2004). Thus, these two hosts display uniquely different silencing response to TMV infection. However, within the phloem it is important to note that TMV induced reductions in silencing components, DCL 3 and AGO 6, that impact the production and function of phloem mobile 24 nt

sRNAs is maintained. Thus, while vsiRNA responses may vary between these hosts the impact of TMV on the phloem mobile sRNAs is conserved, suggesting the virus is targeting the disruption of these systemic signaling RNAs.

Other important pathways involved in plant defenses included ROS metabolic processes. Genes involved in ROS metabolic processes were all down regulated or not affected in Arabidopsis and predominately up regulated in *N. benthamiana*. Genes up regulated in *N. benthamiana* included NADPH oxidases, respiratory burst oxidase homologs (RBOH) D and F, both significant contributors to ROS production during plant defense and stress responses, including pathogen and effector triggered immunities (Suzuki et al., 2011; Willems et al., 2016). The selective up regulation of these oxidases in *N. benthamiana* and not Arabidopsis corresponds with the development of TMV induced systemic necrosis in this host. Four ROS associated genes were down regulated in both hosts (Fig. 8C). Of these one, NEET, is speculated to function in the repression of ROS and has been linked to the activation of plant defense pathways during systemic alfalfa mosaic virus infections (Aparicio and Pallás, 2017; Nechushtai et al., 2012). Reduction of NEET transcripts in all three *N. benthamiana* translatomes is consistent with the observed ROS activation and development of necrotic symptoms. However, reduced NEET levels in the Arabidopsis p35S and pSUC2 translatomes did not correspond with ROS activation, suggesting that in this host TMV can suppress ROS or that wild-type levels of NEET within the pSULTR2;2 associated phloem tissues are sufficient to prevent ROS activation.

Genes involved in SAR also showed a greater response to TMV in *N. benthamiana* than in Arabidopsis. In contrast to the shared up regulation of EARLI1 as described above the majority of SAR related genes were up regulated in *N. benthamiana* while being down regulated or not altered in Arabidopsis (Fig. 9). This included NPR1 and NPR3 like genes that were up regulated

in all tissues of *N. benthamiana* but were not altered in Arabidopsis. NPR1 is a key regulator of the SAR pathway while NPR3 along with NPR4 acts as a receptor for the immune signal salicylic acid (Fu et al., 2012; Moreau et al., 2012). EDS1 and PAD4, which promote SA biosynthesis and are important for maintaining SA-related resistance, are additionally both up regulated in *N. benthamiana* but not altered in Arabidopsis (Cui et al., 2017). From these findings it is clear that *N. benthamiana* mounts a substantially more robust SAR response to TMV infection than does Arabidopsis. However, it is not yet clear if this SAR response is a direct factor in the induction of systemic necrosis in *N. benthamiana* or occurs as a consequence of this host response.

The combined results from these studies reveal for the first time the significant impact TMV infection has on phloem associated tissues. The degree to which the vascular tissue was impacted, in comparison to non-phloem tissues was unexpected and suggests that vascular tissue serves as a key response tissue during infection. In addition, viruses are dependent upon the vascular phloem in order to move systemically, thus many of the host translatome responses observed in this study likely result from the reprogramming of this tissue in order to facilitate virus movement and suppress host defense responses. Previous studies have shown that the TMV replicase protein interacts with and disrupts the function of a phloem CC expressed Aux/IAA transcriptional regulator and that this interaction contributes to efficient virus phloem loading (Collum et al., 2016). Of the transcription factors identified in this study as displaying altered responses in the phloem over 60% contained auxin responsive transcription binding elements in their promoters, indicating they could be impacted through the directed disruption of this Aux/IAA regulator. In summary, this study demonstrates the considerable variation in cell and tissue specific responses that occur during virus infections as well as the importance of spatially dissecting viral – host interactions within whole tissues.

Acknowledgements:

We thank Professor Bailey-Serres, University of California Riverside for providing the translatome constructs. This work was supported in part by grants from the NSF Division of Integrative Organismal Systems (ISO-1120044) and the USDA National Institute of Food and Agriculture, Plant-Associated Microbes and Plant-Microbe Interactions Program (2015-67013-23004). T.D.C. was additionally supported by NIH Institutional Training grant 5T32AI051967 awarded to the University of Maryland.

Figure Legends

Figure 1. Characterization of *promoter:HF-RPL18* transgenic plants. (A) *HF-RPL18* transgene expression in T2 transgenic plants relative to the expression of endogenous *AtRPL18* in non-transformed *A. thaliana*. Quantitative RT-PCR analysis was performed with a primer set specific to *HF-RPL18* or *AtRPL18*, *18S rRNA* was used as the internal control. Bars represent the average of two biological replicates ± standard error. (B) Representative photographs of four week old control and transgenic plants. All *promoter::HF-RPL18* transgenic plant lines display no developmental phenotypes. (C) Histochemical analysis of pSULTR2;2::GUS and pSUC2::GUS in *N. benthamiana* 5-wk-old leaves and petioles after staining overnight. X, xylem; abP, abaxial phloem; adP, adaxial phloem. Scale bars, leaves 1 mm, petioles 200 μm.

Figure 2. TMV accumulation and disease symptoms.

(A) Tissue print immunoblot of TMV infected or mock infected leaves at 6 days post infection (dpi). Black staining indicates TMV CP accumulation. (B) Representative images of TMV or mock infected plants 6 dpi. (C) Representative images of TMV or mock infected plants at 24 dpi.

Figure 3. Tissues specific comparisons of differentially expressed genes in *A. thaliana* and *N. benthamiana*.

Number of unique and shared genes altered 2 to 10-fold in response to TMV in *A. thaliana* (A) or *N. benthamiana* (B). Number of unique and shared genes altered > 10-fold in response to TMV in *A. thaliana* (C) or *N. benthamiana* (D). Genes were characterized into phloem associated (yellow); gene alterations that occurred only in one or both of the two phloem (pSUC2 and pSULTR2;2) translatomes, non-phloem associated (blue); changes that occurred only in the p35S translatome and whole leaf tissues (green); alterations found in at least one of the phloem translatomes and the p35S translatome.

Figure 4. Characteristics of differentially expressed genes of *A. thaliana* and *N. benthamiana* in response to TMV infection.

Number of genes up regulated or down regulated 2 to 10-fold in response to TMV in *A. thaliana* (A) or *N. benthamiana* (B). Number of genes up regulated or down regulated > 10-fold in response to TMV in *A. thaliana* (C) or *N. benthamiana* (D).

Figure 5. Enriched GO biological processes shared between hosts

Heatmaps showing significantly enriched (FDR p-value < 0.05) GO biological processes among up regulated (orange) or down regulated (blue) genes in phloem, whole leaf, and non-phloem tissues in *A. thaliana* (At) and *N. benthamiana* (Nb). Darker shading indicates higher statistical significance of GO term enrichment and white indicates no statistical significance. (A) GO terms enriched among similarly regulated genes. (B) GO terms enriched among oppositely regulated genes.

Figure 6: Phloem transcription factors altered in response to TMV infection.

Heatmap showing phloem transcription factor gene expression in *A. thaliana* and *N. benthamiana*. Changes of mRNA translatome accumulation in TMV infected samples are shown relative to the mRNA translatome accumulation in mock infected samples. Fold-changes of down regulation (blue) or up regulation (red) are shown on a log2 scale. Locus ID numbers for each gene are provided in Table S3.

Figure 7: Lipid localization genes altered in response to TMV infection.

Heatmap showing changes in gene expression for lipid localization genes in *A. thaliana* and *N. benthamiana*. Changes of mRNA translatome accumulation in TMV infected samples are shown relative to the mRNA translatome accumulation in mock infected samples. Fold-changes of down regulation (blue) or up regulation (red) are shown on a log2 scale. Locus ID numbers for each gene are provided in Table S3.

Figure 8: Viral defense pathways altered in response to TMV infection.

Heatmap showing changes in gene expression for (A) RNA silencing pathway genes, (B) Callose synthase genes, (C) Reactive oxygen species (ROS) metabolic processes associated genes in *A. thaliana* and *N. benthamiana*. Changes of mRNA translatome accumulation in TMV infected samples are shown relative to the mRNA translatome accumulation in mock infected samples. Fold-changes of down regulation (blue) or up regulation (red) are shown on a log2 scale. Locus ID numbers for each gene are provided in Table S3.

Figure 9: Systemic acquired resistance genes altered in response to TMV infection.

Heatmap showing systemic acquired resistance related gene expression in *A. thaliana* and *N. benthamiana*. Changes of mRNA translatome accumulation in TMV infected samples are shown relative to the mRNA translatome accumulation in mock infected samples. Fold-changes of down regulation (blue) or up regulation (red) are shown on a log2 scale. Locus ID numbers for each gene are provided in Table S3.

Figure S1. Reads mapped to the TMV genome.

Dark shading indicates number of reads mapped to the TMV genome for each translatome. The TMV genome from 1 to 6395 nucleotides (nt) is represented along the x-axis with the omega (Ω) sequence labeled with a black bar. Number of reads mapped are shown on a scale of 0 to 500 for *A. thaliana* and 0 to 4000 for *N. benthamiana*.

Figure S2. Pairwise comparison of biological replicates in *A. thaliana*.

Scatter plots and Pearson product-moment correlation coefficients (r) comparing two biological replicates for each of the six experimental groups. RPKM, Reads Per Kilobase of transcript per Million mapped reads.

Figure S3. Pairwise comparison of biological replicates in *N. benthamiana*.

Scatter plots and Pearson product-moment correlation coefficients (r) comparing two biological replicates for each of the six experimental groups. RPKM, Reads Per Kilobase of transcript per Million mapped reads.

References

Aparicio, F., Pallás, V., 2017. The coat protein of Alfalfa mosaic virus interacts and interferes with the transcriptional activity of the bHLH transcription factor ILR3 promoting salicylic acid-dependent defence signalling response. Mol Plant Pathol 18, 173-186.

Asahina, M., Azuma, K., Pitaksaringkarn, W., Yamazaki, T., Mitsuda, N., Ohme-Takagi, M., Yamaguchi, S., Kamiya, Y., Okada, K., Nishimura, T., 2011. Spatially selective hormonal control of RAP2. 6L and ANAC071 transcription factors involved in tissue reunion in Arabidopsis. Proc Natl Acad Sci USA 108, 16128-16132.

Baggerly, K.A., Deng, L., Morris, J.S., Aldaz, C.M., 2003. Differential expression in SAGE: accounting for normal between-library variation. Bioinformatics 19, 1477-1483.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 57, 289-300.

Benyon, L.S., Stover, E., Bowman, K.D., Niedz, R., Shatters, R.G., Zale, J., Belknap, W., 2013. GUS expression driven by constitutive and phloem-specific promoters in citrus hybrid US-802. In Vitro Cell Dev Biol Plant 49, 255-265.

Bombarely, A., Rosli, H.G., Vrebalov, J., Moffett, P., Mueller, L.A., Martin, G.B., 2012. A draft genome sequence of Nicotiana benthamiana to enhance molecular plant-microbe biology research. Mol Plant Microbe Interact 25, 1523-1530.

Burch-Smith, T.M., Zambryski, P.C., 2016. Regulation of plasmodesmal transport and modification of plasmodesmata during development and following infection by viruses and viral proteins, Plant-Virus Interactions. Springer, pp. 87-122.

Callaway, A., Giesman-Cookmeyer, D., Gillock, E., Sit, T., Lommel, S., 2001. The multifunctional capsid proteins of plant RNA viruses. Annu Rev Phytopathol 39, 419-460.

Carbonell, A., Carrington, J.C., 2015. Antiviral roles of plant ARGONAUTES. Curr Opin Plant Biol 27, 111-117.

Carella, P., Wilson, D.C., Kempthorne, C.J., Cameron, R.K., 2016. Vascular Sap Proteomics: Providing Insight into Long-Distance Signaling during Stress. Front Plant Sci 7, 651.

Carrington, J.C., Kasschau, K.D., Mahajan, S.K., Schaad, M.C., 1996. Cell-to-Cell and Long-Distance Transport of Viruses in Plants. Plant Cell 8, 1669-1681.

Cecchini, N.M., Steffes, K., Schlappi, M.R., Gifford, A.N., Greenberg, J.T., 2015. Arabidopsis AZI1 family proteins mediate signal mobilization for systemic defence priming. Nat Commun 6, 7658.

Chen, L., Zhang, L., Li, D., Wang, F., Yu, D., 2013. WRKY8 transcription factor functions in the TMV-cg defense response by mediating both abscisic acid and ethylene signaling in Arabidopsis. Proc Natl Acad Sci USA 110, E1963-1971.

Clough, S.J., Bent, A.F., 1998. Floral dip: a simplified method forAgrobacterium-mediated transformation of Arabidopsis thaliana. Plant J 16, 735-743.

Collum, T.D., Padmanabhan, M.S., Hsieh, Y.-C., Culver, J.N., 2016. Tobacco mosaic virus-directed reprogramming of auxin/indole acetic acid protein transcriptional responses enhances virus phloem loading. Proc Natl Acad Sci USA 113, E2740-E2749.

Cui, H., Gobbato, E., Kracher, B., Qiu, J., Bautor, J., Parker, J.E., 2017. A core function of EDS1 with PAD4 is to protect the salicylic acid defense sector in Arabidopsis immunity. New Phytol 213, 1802-1817.

Cui, W., Lee, J.-Y., 2016. Arabidopsis callose synthases CalS1/8 regulate plasmodesmal permeability during stress. Nat Plants 2, 16034.

Czech, B., Hannon, G.J., 2011. Small RNA sorting: matchmaking for Argonautes. Nat Rev Genet 12, 19-31.

Dardick, C.D., Golem, S., Culver, J.N., 2000. Susceptibility and symptom development in Arabidopsis thaliana to Tobacco mosaic virus is influenced by virus cell-to-cell movement. Mol Plant Microbe In 13, 1139-1144.

Davière, J.-M., Achard, P., 2013. Gibberellin signaling in plants. Development 140, 1147-1151.

Davière, J.-M., Achard, P., 2016. A pivotal role of DELLAs in regulating multiple hormone signals. Mol Plant 9, 10-20.

De Bruyne, L., Höfte, M., De Vleesschauwer, D., 2014. Connecting growth and defense: the emerging roles of brassinosteroids and gibberellins in plant innate immunity. Mol Plant 7, 943-959.

De Vos, M., Denekamp, M., Dicke, M., Vuylsteke, M., Van Loon, L., Smeekens, S.C., Pieterse, C., 2006. The Arabidopsis thaliana transcription factor AtMYB102 functions in defense against the insect herbivore Pieris rapae. Plant Signal Behav 1, 305-311.

Deeken, R., Ache, P., Kajahn, I., Klinkenberg, J., Bringmann, G., Hedrich, R., 2008. Identification of Arabidopsis thaliana phloem RNAs provides a search criterion for phloem-based transcripts hidden in complex datasets of microarray experiments. Plant J 55, 746-759.

Deleris, A., Gallego-Bartolome, J., Bao, J.S., Kasschau, K.D., Carrington, J.C., Voinnet, O., 2006. Hierarchical action and inhibition of plant Dicer-like proteins in antiviral defense. Science 313, 68-71.

Denekamp, M., Smeekens, S.C., 2003. Integration of wounding and osmotic stress signals determines the expression of the AtMYB102 transcription factor gene. Plant Physiol 132, 1415-1423.

Du, Z., Zhou, X., Ling, Y., Zhang, Z., Su, Z., 2010. agriGO: a GO analysis toolkit for the agricultural community. Nucleic Acids Res 38, W64-W70.

Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C., Lepiniec, L., 2010. MYB transcription factors in Arabidopsis. Trends Plant Sci 15, 573-581.

Fu, Z.Q., Dong, X., 2013. Systemic acquired resistance: turning local infection into global defense. Annu Rev Plant Biol 64, 839-863.

Fu, Z.Q., Yan, S., Saleh, A., Wang, W., Ruble, J., Oka, N., Mohan, R., Spoel, S.H., Tada, Y., Zheng, N., 2012. NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. Nature 486, 228-232.

Garcia-Ruiz, H., Takeda, A., Chapman, E.J., Sullivan, C.M., Fahlgren, N., Brempelis, K.J., Carrington, J.C., 2010. Arabidopsis RNA-dependent RNA polymerases and dicer-like proteins in antiviral defense and small interfering RNA biogenesis during Turnip Mosaic Virus infection. Plant Cell 22, 481-496.

Gibbs, A.J., 1976. Viruses and plasmodesmata. In: Gunning, B.E.S., Robards, A.W. (Eds.), Intercellular Communication in Plants: Studies on Plasmodesmata, Springer-Verlag, Berlin, pp. 149-164.

Goelet, P., Lomonossoff, G., Butler, P., Akam, M., Gait, M., Karn, J., 1982. Nucleotide sequence of tobacco mosaic virus RNA. Procs Natl Acad Sci USA 79, 5818-5822.

Goodin, M.M., Zaitlin, D., Naidu, R.A., Lommel, S.A., 2008. Nicotiana benthamiana: Its history and future as a model for plant-pathogen interactions. Mol. Plant-Microbe Interact. 21, 1015-1026.

Goodrick, B.J., Kuhn, C.W., Hussey, R.S., 1991. Restricted Systemic Movement of Cowpea Chlorotic Mottle Virus in Soybean with Nonnecrotic Resistance. Phytopathol 81, 1426-1431.

Hedrich, R., 2012. Ion channels in plants. Physiol Rev 92, 1777-1811.

Heinlein, M., 2015. Plasmodesmata: channels for viruses on the move. Methods Mol Biol 1217, 25-52.

Hipper, C., Brault, V., Ziegler-Graff, V., Revers, F., 2013. Viral and cellular factors involved in phloem transport of plant viruses. Front Plant Sci 4, 154.

Holsters, M., De Waele, D., Depicker, A., Messens, E., Van Montagu, M., Schell, J., 1978. Transfection and transformation of Agrobacterium tumefaciens. Mol Gen Genet 163, 181-187.

Horsch, R.B., Fry, J.E., Hoffman, N.L., Eicholtz, D.A., Rogers, S.G., Fraley, R.T., 1985. A simple and general method for transferring genes into plants. Science 227, 1229-1231.

Hou, X., Ding, L., Yu, H., 2013. Crosstalk between GA and JA signaling mediates plant growth and defense. Plant Cell Rep 32, 1067-1074.

Iglesias, V.A., Meins, F., 2000. Movement of plant viruses is delayed in a β-1, 3-glucanase-deficient mutant showing a reduced plasmodesmatal size exclusion limit and enhanced callose deposition. The Plant Journal 21, 157-166.

Isaacs, M., Carella, P., Faubert, J., Rose, J.K., Cameron, R.K., 2016. Orthology Analysis and In Vivo Complementation Studies to Elucidate the Role of DIR1 during Systemic Acquired Resistance in Arabidopsis thaliana and Cucumis sativus. Front Plant Sci 7, 566.

Kay, R., Chan, A., Daly, M., McPherson, J., 1987. Duplication of CaMV 35S promoter sequences creates a strong enhancer for plant genes. Science 236, 1299-1302.

Knecht, D.A., Dimond, R.L., 1984. Visualization of antigenic proteins on Western blots. Anal Biochem 136, 180-184.

Knoblauch, M., Oparka, K., 2012. The structure of the phloem--still more questions than answers. Plant J 70, 147-156.

Krizek, B., 2009. AINTEGUMENTA and AINTEGUMENTA-LIKE6 act redundantly to regulate Arabidopsis floral growth and patterning. Plant Physiol 150, 1916-1929.

Krizek, B.A., Bequette, C.J., Xu, K., Blakley, I.C., Fu, Z.Q., Stratmann, J., Loraine, A.E., 2016. RNA-Seq links AINTEGUMENTA and AINTEGUMENTA-LIKE6 to cell wall remodeling and plant defense pathways in Arabidopsis. Plant Physiol, pp. 01625.02015.

Lamesch, P., Berardini, T.Z., Li, D., Swarbreck, D., Wilks, C., Sasidharan, R., Muller, R., Dreher, K., Alexander, D.L., Garcia-Hernandez, M., 2012. The Arabidopsis Information Resource (TAIR): improved gene annotation and new tools. Nucleic Acids Res 40, D1202-D1210.

Levy, A., Zheng, J.Y., Lazarowitz, S.G., 2013. The tobamovirus Turnip Vein Clearing Virus 30-kilodalton movement protein localizes to novel nuclear filaments to enhance virus infection. J Virol 87, 6428-6440.

Li, W., Zhao, Y., Liu, C., Yao, G., Wu, S., Hou, C., Zhang, M., Wang, D., 2012. Callose deposition at plasmodesmata is a critical factor in restricting the cell-to-cell movement of Soybean mosaic virus. Plant Cell Rep 31, 905-916.

Lin, S.-Y., Chen, P.-W., Chuang, M.-H., Juntawong, P., Bailey-Serres, J., Jauh, G.-Y., 2014. Profiling of translatomes of in vivo–grown pollen tubes reveals genes with roles in micropylar guidance during pollination in arabidopsis. Plant Cell 26, 602-618.

Liu, C., Nelson, R.S., 2013. The cell biology of Tobacco mosaic virus replication and movement. Front Plant Sci 4, 12.

Lucas, W.J., Groover, A., Lichtenberger, R., Furuta, K., Yadav, S.R., Helariutta, Y., He, X.Q., Fukuda, H., Kang, J., Brady, S.M., Patrick, J.W., Sperry, J., Yoshida, A., Lopez-Millan, A.F., Grusak, M.A., Kachroo, P., 2013. The plant vascular system: evolution, development and functions. J Integr Plant Biol 55, 294-388.

Mallory, A., Vaucheret, H., 2010. Form, Function, and Regulation of ARGONAUTE Proteins. Plant Cell 22, 3879-3889.

Melnyk, C.W., Molnar, A., Baulcombe, D.C., 2011. Intercellular and systemic movement of RNA silencing signals. Embo Journal 30, 3553-3563.

Molnar, A., Melnyk, C.W., Bassett, A., Hardcastle, T.J., Dunn, R., Baulcombe, D.C., 2010. Small Silencing RNAs in Plants Are Mobile and Direct Epigenetic Modification in Recipient Cells. Science 328, 872-875.

Moreau, M., Tian, M., Klessig, D.F., 2012. Salicylic acid binds NPR3 and NPR4 to regulate NPR1-dependent defense responses. Cell Res 22, 1631-1633.

Mortazavi, A., Williams, B.A., McCue, K., Schaeffer, L., Wold, B., 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods 5, 621-628.

Mustroph, A., Zanetti, M.E., Jang, C.J., Holtan, H.E., Repetti, P.P., Galbraith, D.W., Girke, T., Bailey-Serres, J., 2009. Profiling translatomes of discrete cell populations resolves altered cellular priorities during hypoxia in Arabidopsis. Proc Natl Acad Sci USA 106, 18843-18848.

Nechushtai, R., Conlan, A.R., Harir, Y., Song, L., Yogev, O., Eisenberg-Domovich, Y., Livnah, O., Michaeli, D., Rosen, R., Ma, V., 2012. Characterization of Arabidopsis NEET reveals an ancient role for NEET proteins in iron metabolism. Plant Cell 24, 2139-2154.

Nelson, R.S., van Bel, A.J., 1998. The mystery of virus trafficking into, through and out of vascular tissue, Prog Bot 59, 476-533.

Qi, X.P., Bao, F.S., Xie, Z.X., 2009. Small RNA Deep Sequencing Reveals Role for Arabidopsis thaliana RNA-Dependent RNA Polymerases in Viral siRNA Biogenesis. PLoS One 4, e4971.

Reynoso, M.A., Juntawong, P., Lancia, M., Blanco, F.A., Bailey-Serres, J., Zanetti, M.E., 2015. Translating Ribosome Affinity Purification (TRAP) followed by RNA sequencing technology (TRAP-SEQ) for quantitative assessment of plant translatomes. Methods Mol Biol 1284, 185-207.

Santa Cruz, S., 1999. Perspective: phloem transport of viruses and macromolecules - what goes in must come out. Trends Microbiol 7, 237-241.

Sasaki, N., Ogata, T., Deguchi, M., Nagai, S., Tamai, A., Meshi, T., Kawakami, S., Watanabe, Y., Matsushita, Y., Nyunoya, H., 2009. Over-expression of putative transcriptional coactivator KELP interferes with Tomato mosaic virus cell-to-cell movement. Mol Plant Pathol 10, 161-173.

Scholthof, H.B., 2005. Plant virus transport: motions of functional equivalence. Trends Plant Sci 10, 376-382.

Stadler, R., Sauer, N., 1996. The Arabidopsis thaliana AtSUC2 gene is specifically expressed in companion cells. Plant Biol 109, 299-306.

Sun, T.-p., 2011. The molecular mechanism and evolution of the GA–GID1–DELLA signaling module in plants. Curr Biol 21, R338-R345.

Sunilkumar, G., Mohr, L., Lopata-Finch, E., Emani, C., Rathore, K.S., 2002. Developmental and tissue-specific expression of CaMV 35S promoter in cotton as revealed by GFP. Plant Mol Biol 50, 463-479.

Suzuki, N., Miller, G., Morales, J., Shulaev, V., Torres, M.A., Mittler, R., 2011. Respiratory burst oxidases: the engines of ROS signaling. Curr Opin Plant Biol 14, 691-699.

Takahashi, H., Watanabe-Takahashi, A., Smith, F.W., Blake-Kalff, M., Hawkesford, M.J., Saito, K., 2000. The roles of three functional sulphate transporters involved in uptake and translocation of sulphate in Arabidopsis thaliana. The Plant Journal 23, 171-182.

Takeda, A., Iwasaki, S., Watanabe, T., Utsumi, M., Watanabe, Y., 2008. The mechanism selecting the guide strand from small RNA duplexes is different among argonaute proteins. Plant Cell Physiol 49, 493-500.

Truernit, E., Sauer, N., 1995. The promoter of the Arabidopsis thaliana SUC2 sucrose-H+ symporter gene directs expression of β-glucuronidase to the phloem: evidence for phloem loading and unloading by SUC2. Planta 196, 564-570.

Turgeon, R., Wolf, S., 2009. Phloem transport: cellular pathways and molecular trafficking. Annu Rev Plant Biol 60, 207-221.

Turnbull, C.G., Lopez-Cobollo, R.M., 2013. Heavy traffic in the fast lane: long-distance signalling by macromolecules. New Phytol 198, 33-51.

Ueki, S., Citovsky, V., 2002. The systemic movement of a tobamovirus is inhibited by a cadmium-ion-induced glycine-rich protein. Nat Cell Biol 4, 478-486.

Ueki, S., Spektor, R., Natale, D.M., Citovsky, V., 2010. ANK, a host cytoplasmic receptor for the Tobacco mosaic virus cell-to-cell movement protein, facilitates intercellular transport through plasmodesmata. PLoS Pathog 6, e1001201.

van Bel, A.J., 1996. Interaction between sieve element and companion cell and the consequences for photoassimilate distribution. Two structural hardware frames with associated physiological software packages in dicotyledons? J Exp Bot 47, 1129-1140.

Vatén, A., Dettmer, J., Wu, S., Stierhof, Y.-D., Miyashima, S., Yadav, S.R., Roberts, C.J., Campilho, A., Bulone, V., Lichtenberger, R., 2011. Callose biosynthesis regulates symplastic trafficking during root development. Dev Cell 21, 1144-1155.

Wang, A.M., 2015. Dissecting the Molecular Network of Virus-Plant Interactions: The Complex Roles of Host Factors. Annu Rev Phytopathol 53, 45-66.

Wang, X.B., Wu, Q.F., Ito, T., Cillo, F., Li, W.X., Chen, X.M., Yu, J.L., Ding, S.W., 2010. RNAi-mediated viral immunity requires amplification of virus-derived siRNAs in Arabidopsis thaliana. Proc Natl Acad Sci U S A 107, 484-489.

Willems, P., Mhamdi, A., Simon, S., Storme, V., Kerchev, P.I., Noctor, G., Gevaert, K., Van Breusegem, F., 2016. The ROS wheel: refining ROS transcriptional footprints in Arabidopsis. Plant Physiol 171, 1720-1733.

Yang, S.-J., Carter, S.A., Cole, A.B., Cheng, N.-H., Nelson, R.S., 2004. A natural variant of a host RNA-dependent RNA polymerase is associated with increased susceptibility to viruses by Nicotiana benthamiana. Proc Natl Acad Sci USA 101, 6297-6302.

Yilmaz, A., Mejia-Guerra, M.K., Kurz, K., Liang, X., Welch, L., Grotewold, E., 2011. AGRIS: the Arabidopsis gene regulatory information server, an update. Nucleic Acids Res 39, D1118-D1122.

Yu, K.S., Soares, J.M., Mandal, M.K., Wang, C.X., Chanda, B., Gifford, A.N., Fowler, J.S., Navarre, D., Kachroo, A., Kachroo, P., 2013. A Feedback Regulatory Loop between G3P and Lipid Transfer Proteins DIR1 and AZI1 Mediates Azelaic-Acid-Induced Systemic Immunity. Cell Rep 3, 1266-1278.

Zhang, C., Wu, Z.J., Li, Y., Wu, J.G., 2015. Biogenesis, Function, and Applications of Virus-Derived Small RNAs in Plants. Front Microbiol 6, 1-12.

Zhao, C., Craig, J.C., Petzold, H.E., Dickerman, A.W., Beers, E.P., 2005. The xylem and phloem transcriptomes from secondary tissues of the Arabidopsis root-hypocotyl. Plant Physiol 138, 803-818.

Figure 1. Characterization of *promoter:HF-RPL18* transgenic plants.

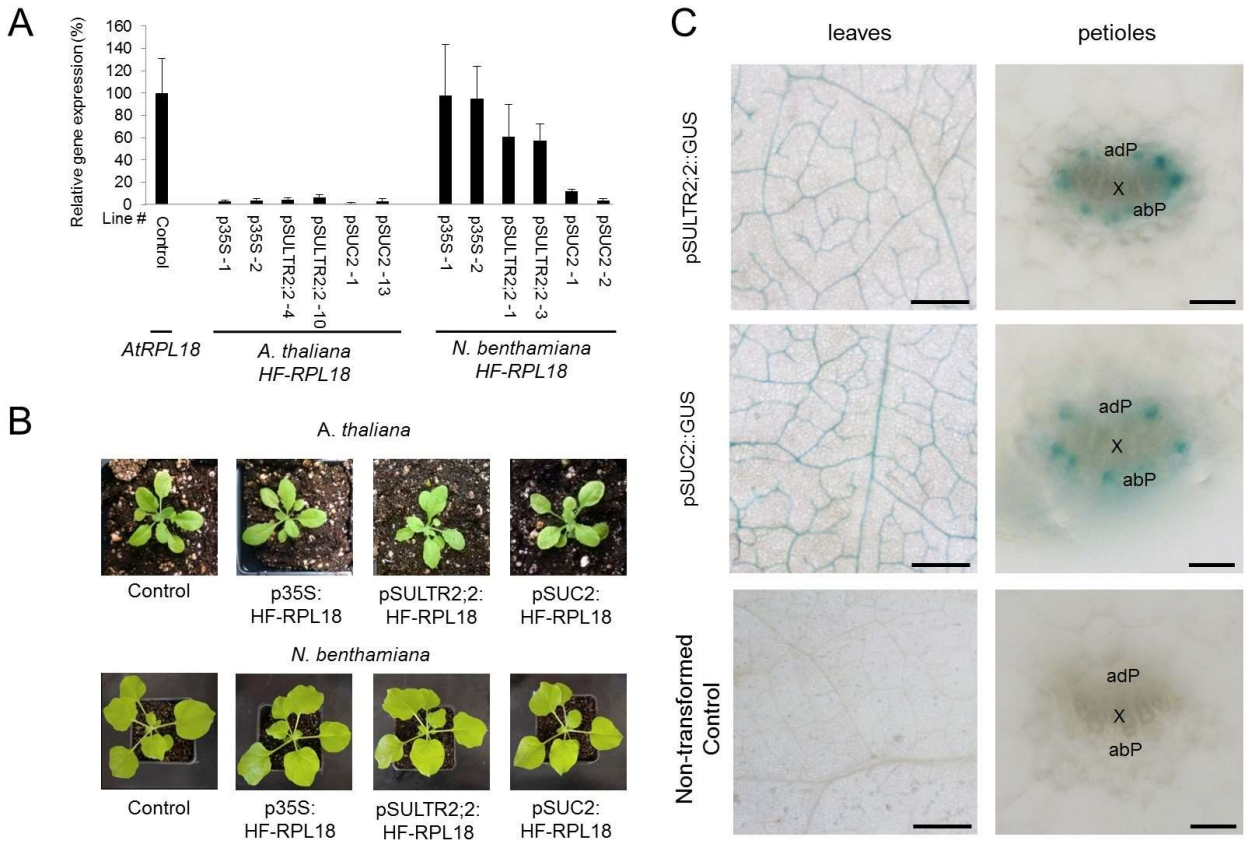

Figure 2. TMV accumulation and disease symptoms.

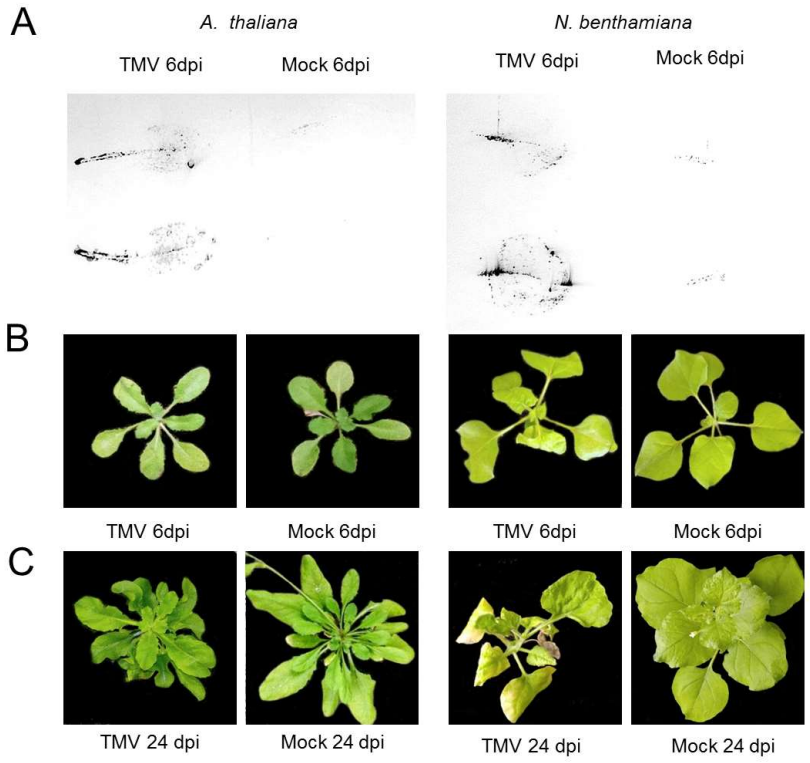

Figure 3. Tissues specific comparisons of differentially expressed genes in *A. thaliana* and *N. benthamiana*.

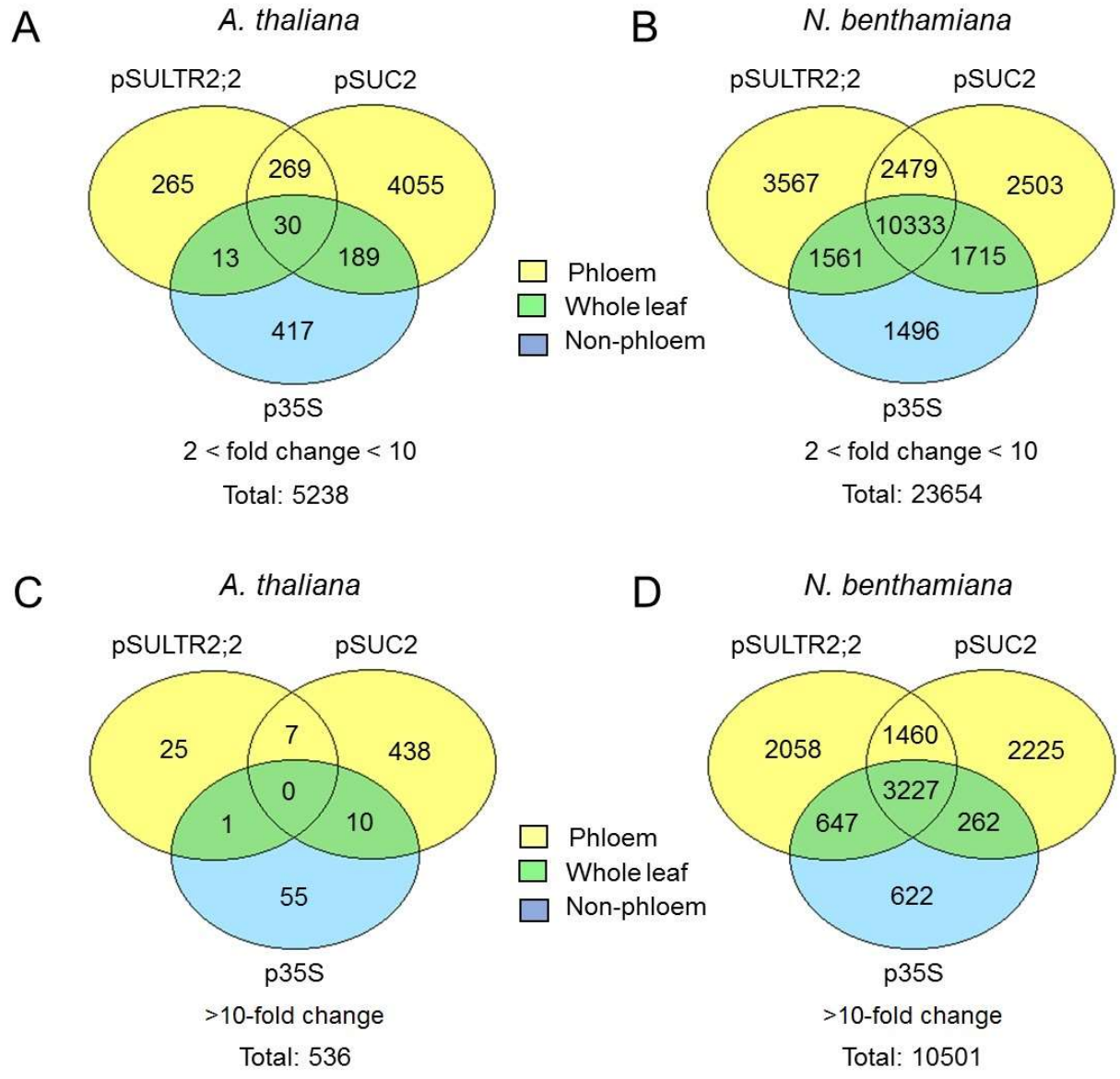

Figure 4. Characteristics of differentially expressed genes of *A. thaliana* and *N. benthamiana* in response to TMV infection.

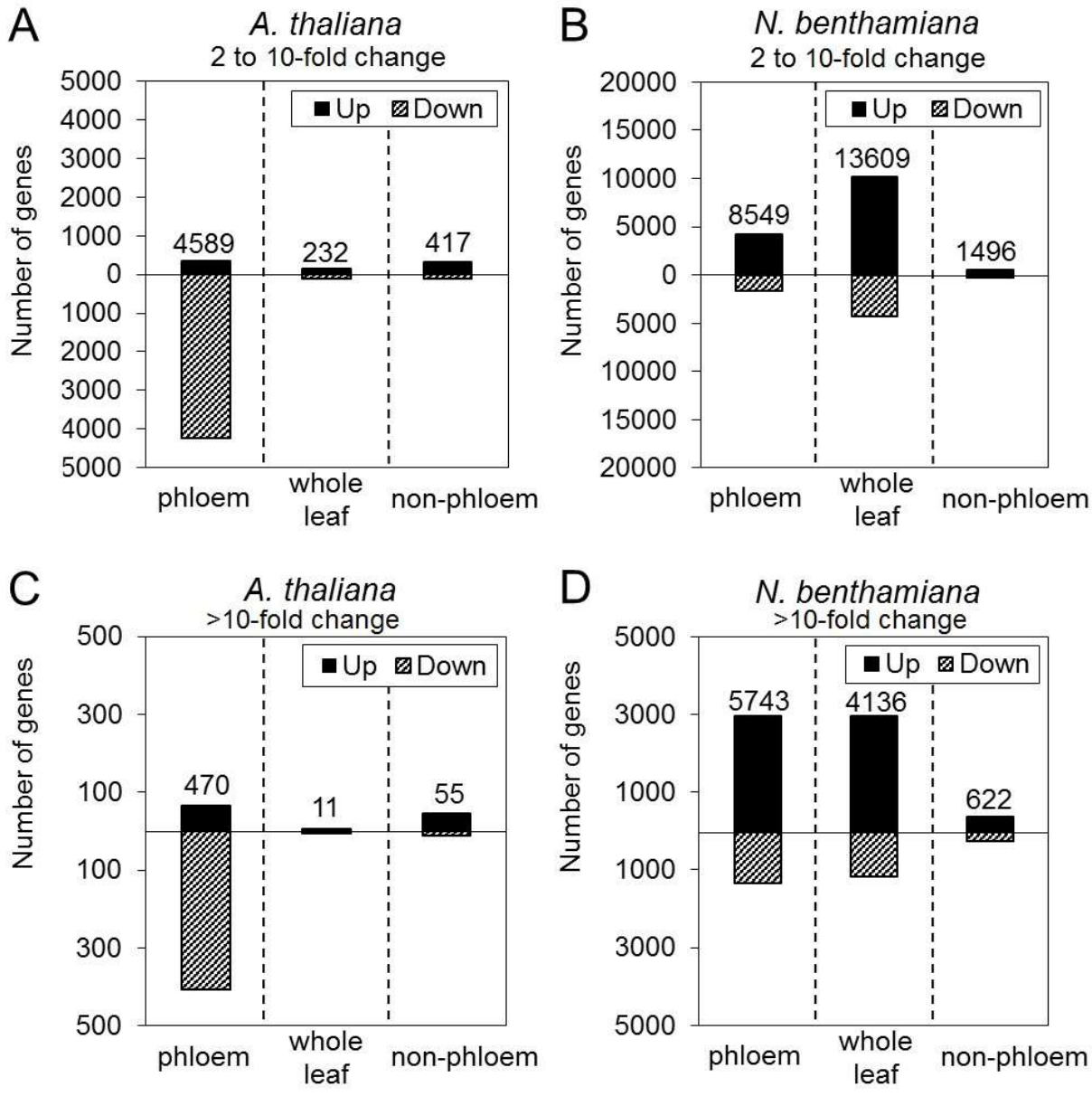

Figure 5. Enriched GO biological processes shared between hosts

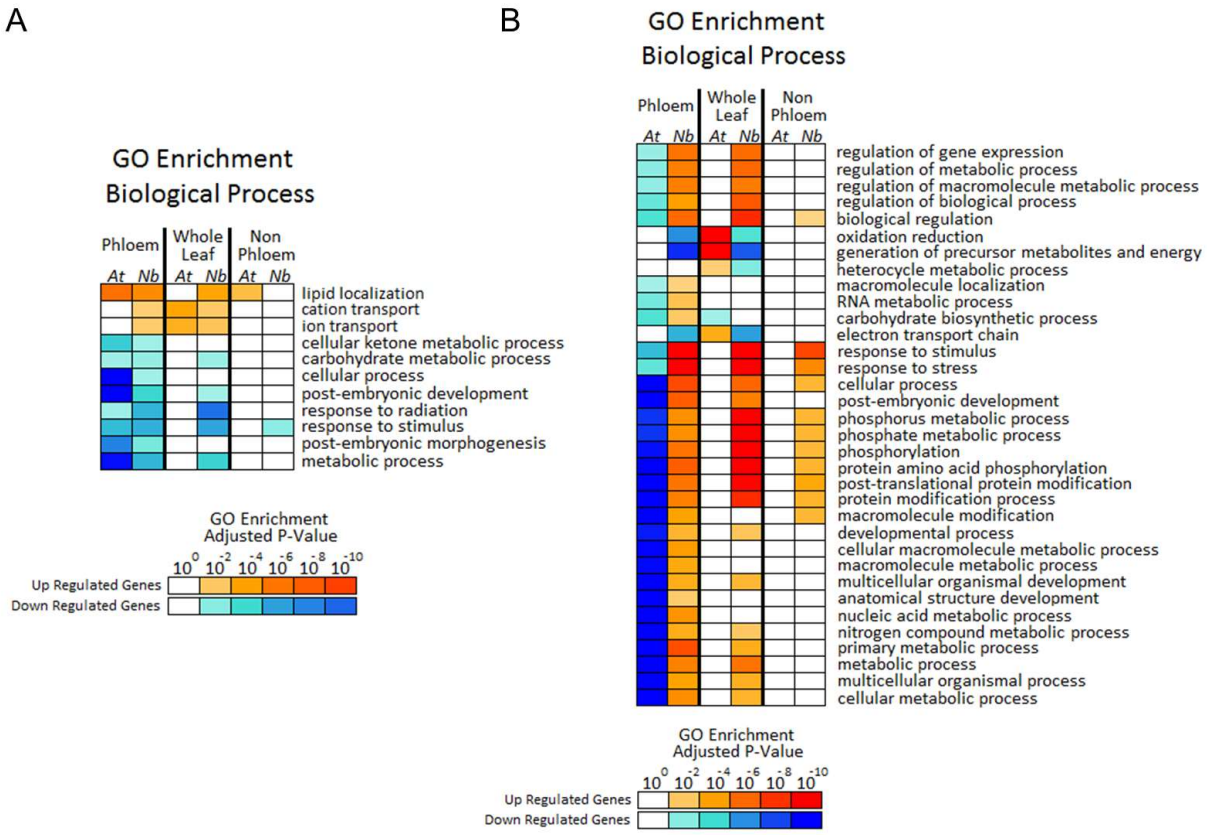

Figure 6: Phloem transcription factors altered in response to TMV infection.

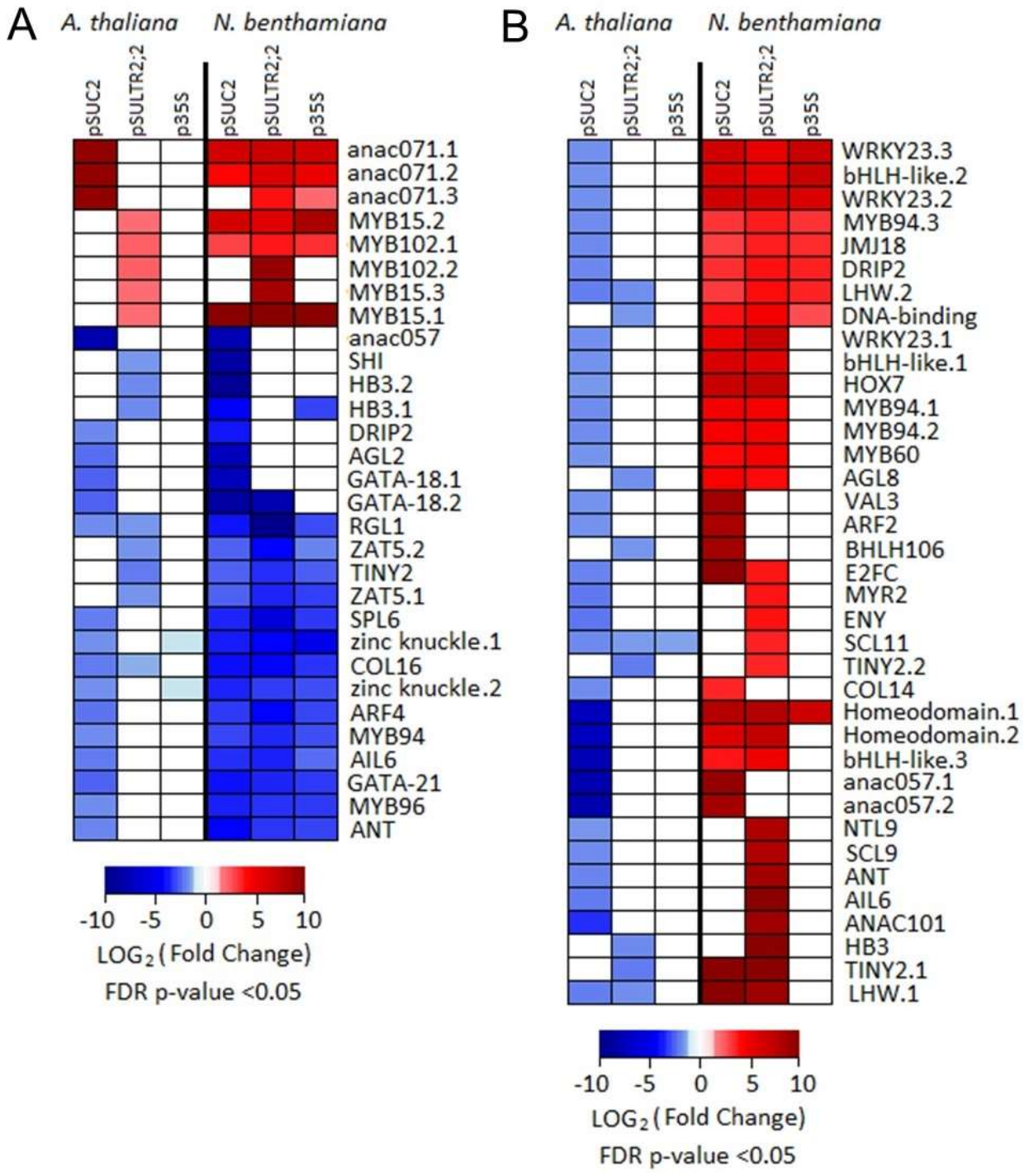

Figure 7: Lipid localization genes altered in response to TMV infection.

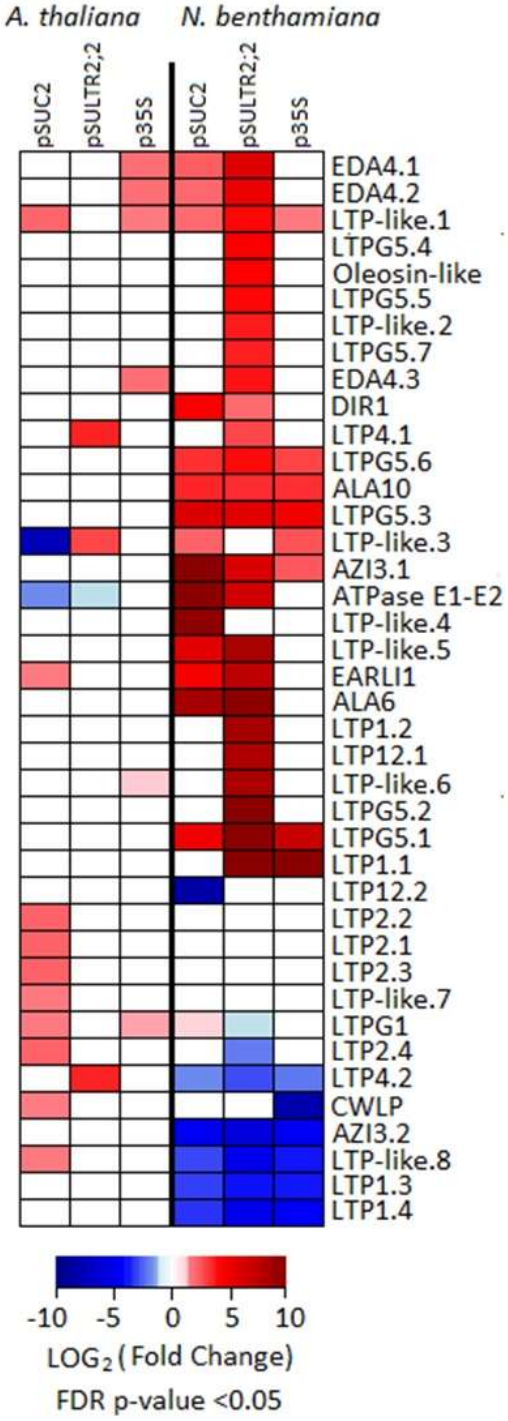

Figure 8: Viral defense pathways altered in response to TMV infection.

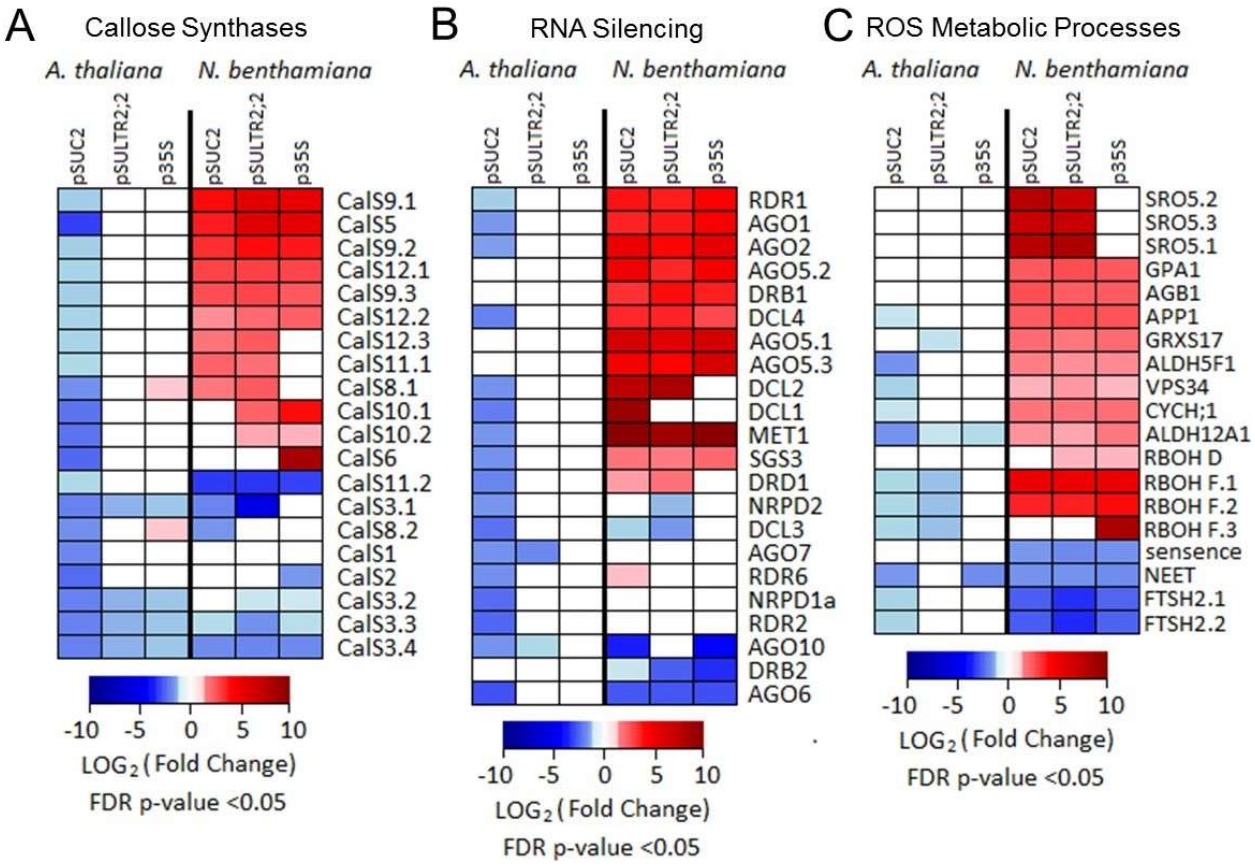

Figure 9: Systemic acquired resistance genes altered in response to TMV infection.

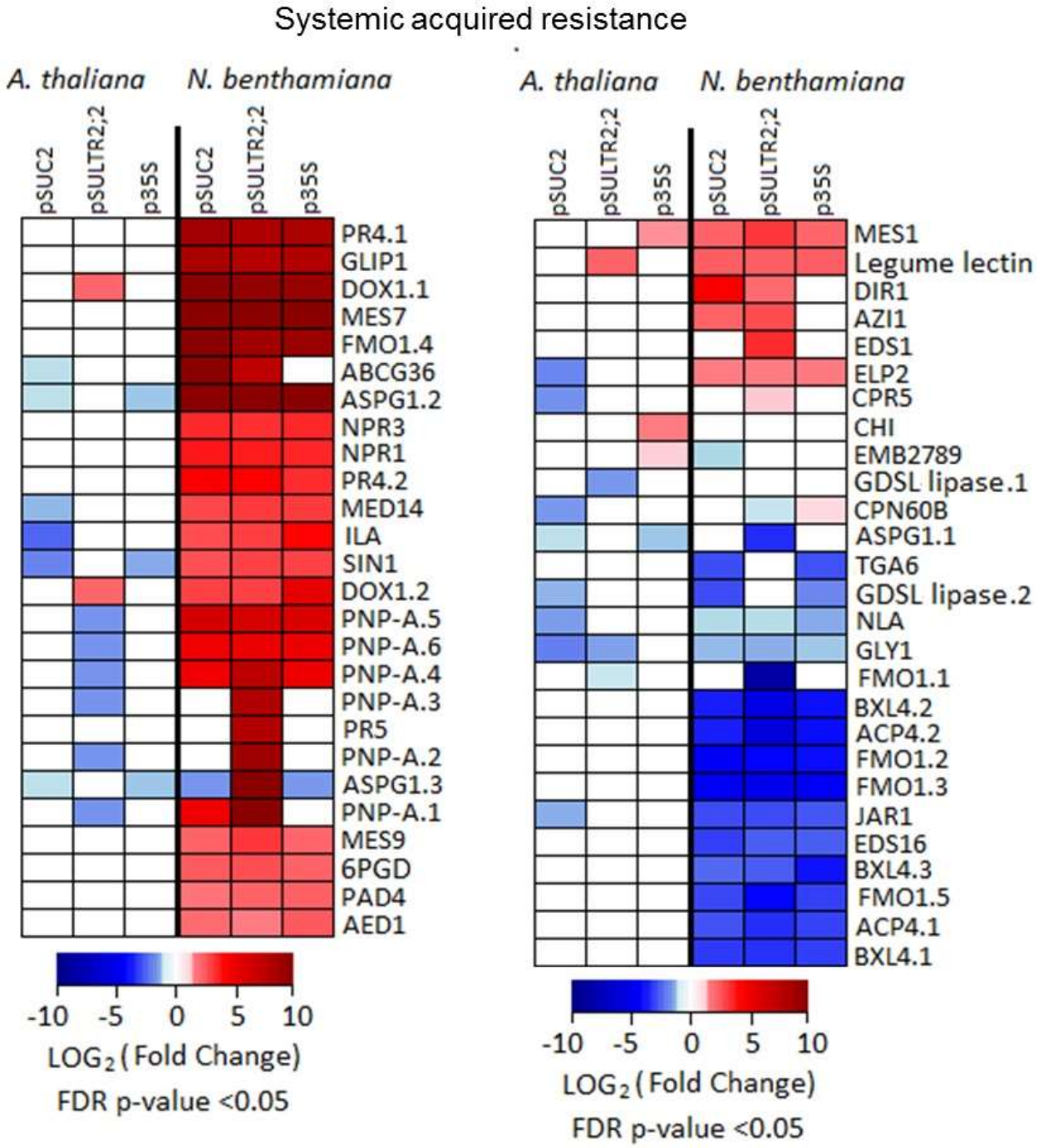

Figure S1. Reads mapped to the TMV genome.

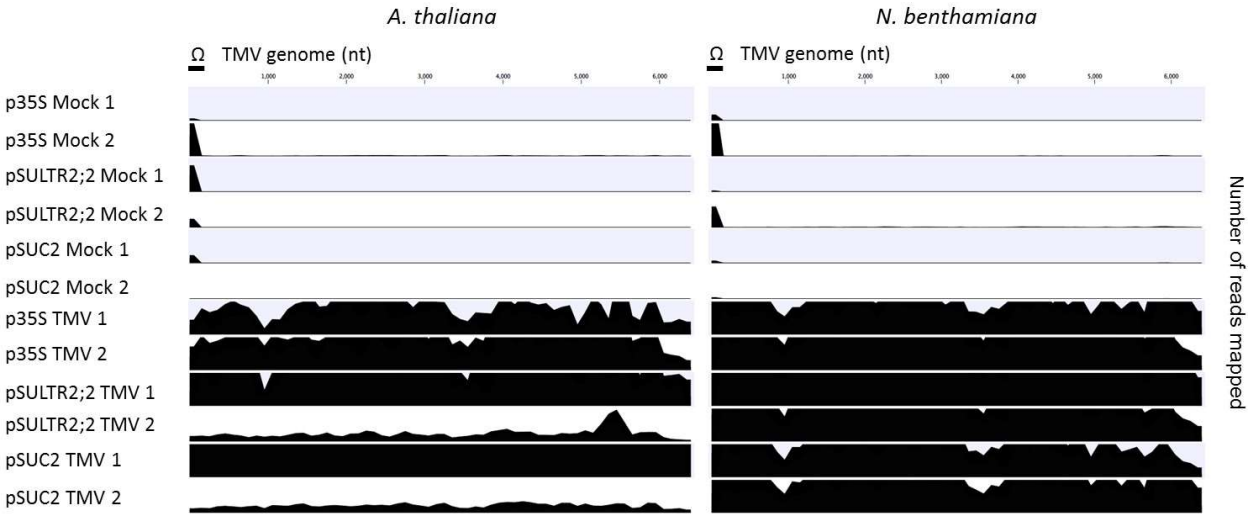

Figure S2. Pairwise comparison of biological replicates in *A. thaliana*.

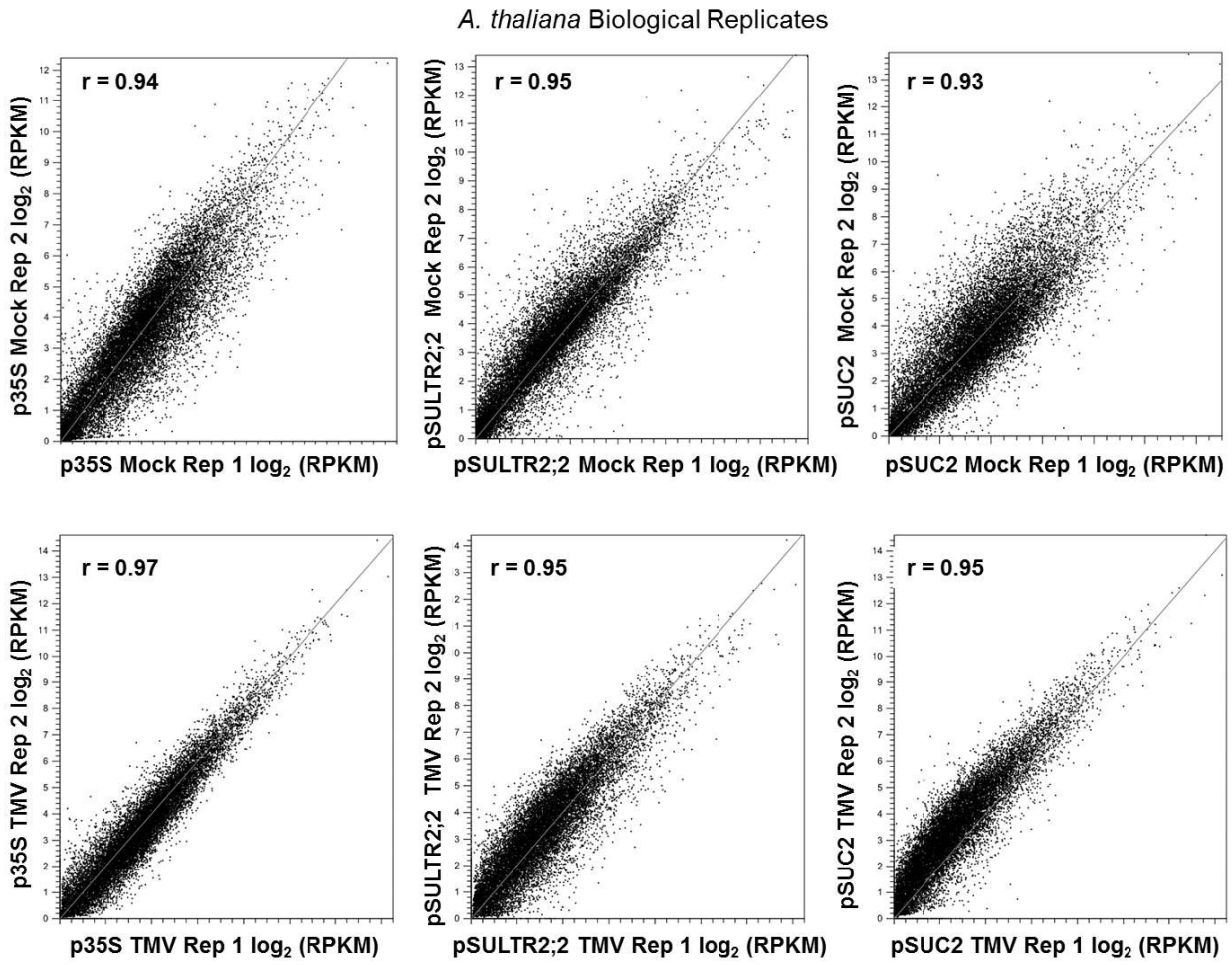

Figure S3. Pairwise comparison of biological replicates in *N. benthamiana*.