Investigation of the effectiveness and molecular mechanisms of thiamin priming to control early blight disease in potato

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Abstract

In several plant species, thiamin foliar application primes plant immunity and can be effective in controlling various diseases. However, the effectiveness of thiamin against potato pathogens has seldom been investigated. Additionally, the transcriptomics and metabolomics of immune priming by thiamin have not previously been investigated. Here, we tested the effect of thiamin application against *Alternaria solani,* the causal agent of early blight in potato, and identified associated changes in gene expression and metabolite content. Thiamin applied on foliage at an optimal concentration of 10 mM reduced lesion size by ~33%. However, prevention of lesion growth was temporally limited, as a reduction of lesion size occurred when leaves were inoculated 4 h, but not 24 h, following thiamin treatment. Additionally, the effect of thiamin on lesion size was restricted to the application site and was not systemic. RNA-seq analysis showed that thiamin affected the expression of 308 genes involved in the synthesis of salicylic acid, secondary metabolites, fatty acid, chitin, primary metabolism and photosynthesis. Genes in these pathways were also amongst the thousands of genes differentially regulated in the response to pathogen alone, though they were often more differentially expressed and enriched when thiamin and the pathogen were combined. Thiamin also delayed the downregulation of photosynthesis-associated genes in plants inoculated with *A. solani*. Metabolite analyses revealed that thiamin treatment in the absence of pathogen decreased the amounts of several organic compounds involved in the citric acid cycle. We hypothesize that thiamin primes plant defenses through perturbation of primary metabolism.

Keywords

Thiamin, potato, *Alternaria solani*, priming, early blight

Background

Early blight of potato is a disease caused by the fungal pathogen *Alternaria solani* (Family *Pleosporaceae*). The primary symptom of early blight is the presence of necrotic lesions on leaves, often most prevalent in senescing or stressed tissue (Abuley and Nielsen 2017). Small circular lesions progress into large angular lesions eventually causing localized death. The ensuing reduced photosynthetic ability can result in a dramatic yield reduction (Rotem 1994). The pathogen can also be symptomatic on tubers, resulting in dry rot symptoms and the formation of dark and sunken lesions on the tuber surface (Waals et al. 2001), making the tuber unmarketable and unsuited for processing. *A. solani* occurs in nearly all potato growing regions of the world and under many different climates (Leiminger and Hausladen 2012). Environmental conditions such as high moisture and temperature can speed the development of the disease (Runno-Paurson et al. 2015; Vloutoglou and Kalogerakis 2000). The conventional approach to controlling early blight in potato is the application of fungicides, which may reduce disease symptoms (Davidson et al. 2016; Horsfield et al. 2010; Rosenzweig et al. 2008), especially when applied at critical times such as late bulking and tuber maturation (Yellareddygari et al. 2019). However, many of the major classes of fungicides used to control early blight have been shown

to lose effectiveness due to fungicide resistance (Fairchild et al. 2013; Rosenzweig et al. 2008). Moreover, fungicides can have lasting negative effects on both the environment and human health (Alavanja et al. 2014; Bozdogan 2014). Due to environmental and health effects, along with the emergence of fungicide-resistant strains of *A. solani,* more management strategies must be investigated.

To manage plant disease, an alternative strategy to fungicides is the application of chemical elicitors for immune priming of plant defenses, characterized by the activation of host plant defense responses, such as transcription of defense genes and the synthesis of phytoalexins, in advance of pathogen infection. Amongst chemical elicitors, B group vitamins have recently received attention as natural plant products that are able to prime plant defenses and reduce disease incidence (Boubakri et al. 2016). Thiamin (vitamin B1), in its pyrophosphorylated form thiamin diphosphate (ThDP), is a cofactor for key enzymes of carbohydrate, amino acid, and fatty acid metabolism (Goyer 2010; Rapala-Kozik 2011). In particular, ThDP is a cofactor for both mitochondrial and chloroplastic pyruvate dehydrogenases, which are involved in glycolysis and *de novo* fatty acid biosynthesis, respectively. ThDP is also a cofactor of transketolase, a key enzyme of the oxidative pentose phosphate pathway and the Calvin cycle. Another key enzyme of primary metabolism that uses ThDP as a cofactor is 2-oxoglutarate dehydrogenase, which has essential roles in the citric/tricarboxylic acid (TCA) cycle, nitrogen assimilation, and amino acid metabolism. ThDP is essential for the synthesis of the branched-chain amino acids valine, leucine, and isoleucine as a cofactor for acetolactate

synthase, and also serves as a cofactor for 1-deoxy-D-xylulose-5-phosphate (DXP) synthase, which synthesizes DXP, a precursor of isoprenoids via the mevalonate-independent pathway.

Thiamin has been shown to prime plant defenses when externally applied to plant foliage in advance of pathogen challenge (Ahn et al. 2005; Ahn et al. 2007; Bahuguna et al. 2012; Huang et al. 2016). Priming of plant defenses with thiamin was demonstrated to slow or stop infections from fungal, viral, and bacterial pathogens in a variety of hosts, including *Arabidopsis*, soybean, rice, grape, tobacco and cucumber (Abdel-Monaim 2011; Ahn et al. 2005; Ahn et al. 2007; Boubakri et al. 2012). Plant defenses triggered by thiamin application include callose deposition, phytoalexin production, pathogenesis-related (PR) gene expression, and production of reactive oxygen species (ROS) (Boubakri et al. 2016). An increase in biosynthesis of secondary metabolites such as terpenoids, phenylpropanoids and antioxidants was found in grapevine upon treatment with thiamin, and the production of such molecules are most likely regulated through molecules such as lipoxygenases, which are also upregulated upon thiamin treatment on grapevine (Boubakri et al. 2012). These molecular changes involve the SA-dependent signaling pathway in *Arabidopsis* (Ahn et al. 2007). In potato, application of thiamin decreased the viral titer of potato virus Y (Vinchesi et al. 2017). However, no other studies have been conducted to address the potential of thiamin as a priming agent in potato against other pathogens. It is also unclear how thiamin primes plant defenses in any of the plant pathosystems studied so far.

In this study, our first objective was to evaluate the effectiveness of thiamin priming treatments against foliar *A. solani* infections in potato. Subsequently, after demonstrating that

thiamin treatment effectively decreased disease symptoms caused by *A. solani*, we analyzed changes of gene expression that occur following thiamin treatment, alone and in the context of pathogen challenge, in potato, utilizing RNA-seq and Gene Ontology (GO) enrichment analysis. We also evaluated thiamin-responsive changes in the metabolome by employing gas chromatography mass spectrometry (GC-MS).

Materials and Methods

Chemicals

Thiamin, Tween 20, pyridine and methoxamine hydrochloride were purchased from Millipore Sigma (Burlington, MA, USA). Methanol and chloroform were purchased from Fisher Scientific (Hampton, NH, USA). N-methyl-N-(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane was purchased from CovaChem (Loves Park, IL, USA).

Plant growth

All experiments were done with the potato variety Russet Norkotah, with one exception as noted below, in which one experiment incorporated the variety Russet Burbank as well; both varieties were chosen because of their susceptibility to early blight. Plantlets were grown for three to four weeks on solid Murashige and Skoog (MS) medium as previously described (Combest et al. 2021), with the exception of utilizing a sucrose concentration of 3%. Plantlets were then transferred to pots containing a mixture of sand and potting soil (Sunshine Mix #4) (Sun Gro Horticulture, Agawam, MA, USA) (v/v 1:4) and slow-release fertilizer (Osmocote Plus, The Scotts Miracle-Gro Company, Marysville, OH, USA). Plants were then grown in greenhouses

until they reached the 9th leaf stage before utilization in experiments; this developmental stage provided leaflets of convenient size for disease assays. Plants received 14 h daily light exposure, and supplementary lighting was provided with 400 W high-pressure sodium lamps. Greenhouse temperature was maintained at 21°C day and 18°C night. For whole plant assays, plants were placed in a 1.22 m x 0.91 m x 2.44 m humidity chamber made from PVC piping and clear plastic purchased from a local retailer.

Thiamin foliar treatments

Thiamin solutions were prepared in deionized water that included Tween 20 at 250 mg L^{-1} to facilitate dispersion of thiamin to the foliage; mock solution consisted of Tween 20 at 250 mg L^{-1} in deionized water. Treatment solutions were sprayed onto foliage via a handheld spray bottle until runoff (approximately 30 mL per plant). Thiamin was applied at the given concentrations (0, 1, 5, 10, 25, 50 mM) and timepoints (4 h, 28 h) prior to pathogen inoculation as noted.

A. solani inoculations

Alternaria solani strain BMP 183 (Andersen et al. 2008) was grown on V8 agar medium plates (Sinclair and Dhingra 1995) from a glycerol stock kept at -80°C. After three days growth, the pathogen was sub-cultured onto fresh V8 agar plates and grown under continuous light until complete coverage of the plate was observed (15-21 days). Plates were then covered with 5 mL deionized water and conidia were gently dislodged using a plastic spreader. Conidia were then transferred into a 50-mL conical tube, vortexed to release spores from mycelium, and filtered through four layers of cheesecloth. Conidia were counted via hemocytometer (Marienfeld Superior, Lauda-Königshofen, Germany). Concentrations ranged between $1.5x10^4$-$3.0x10^4$spores

mL^{-1} for all assays, except in systemic immunity assays, where spore concentration was approximately $6.0x10^3$spores mL^{-1}. Spore concentrations used for each experiment are indicated in figure legends as appropriate.

Detached leaflet inoculations

After mock or thiamin treatment on whole plants, four leaflets were removed using a sterile scalpel from the 3rd and 4th leaves from the top of each plant. Leaflets were rinsed with deionized water to remove any treatment residue and thiamin precipitate and allowed to dry before inoculations. Laboratory wipes (Kimwipe) (2.5 x 2.5 cm) (Kimberly-Clark Professional, Roswell, GA, USA) were wrapped around the petiole of the leaflet. Leaflets were then arranged on pipette tip holders in 1020 garden trays (Greenhouse Megastore, Danville, IL, USA) and separated by treatment group, with one biological replicate per tray; four biological replicates per group were used in all assays. The detached leaflets were then drop-inoculated with four equally spaced drops of 20 µL inocula on the adaxial side of the leaflet. Three leaflets per plant were used for *A. solani* inoculation, and one inoculated with deionized water only as control. After pathogen inoculation, wipe squares were saturated daily with 200 µL deionized water to keep leaflets hydrated. To maintain high humidity for optimum infection, 250 mL of reverse osmosis water was added to the bottom of the trays, which were covered with clear plastic domes (Greenhouse Megastore) and sealed with packing tape. Trays were placed in a dark growth chamber (R.W. Smith & Co., San Diego, CA, USA) immediately post-inoculation for 14 h at 22°C, then under a 10 h photoperiod until lesions were large enough for measurement, at 3-4 days post-inoculation.

To evaluate the efficacy of thiamin as a priming agent for systemic immunity against *A. solani*, whole plants were treated with thiamin with multiple leaves enclosed in plastic zip-lock bags (SC Johnson, Racine, WI, USA) to shield those leaves from direct contact with thiamin. Bagged and unbagged leaves were removed 4 hpt and incorporated in a detached leaf inoculation assay as described above.

For the desensitization assay, whole plants were sprayed with 10 mM thiamin or mock solution, and detached leaflets were inoculated with *A. solani* 4 hpt. After seven additional days, plants were sprayed with a second application of thiamin, and a second set of detached leaflets, that were also previously treated with thiamin or mock solutions, were removed and inoculated with *A. solani*.

Whole plant inoculations

Plants were placed in the humidity chamber, and four to twelve leaflets were inoculated directly on the plant with two to six 10 µL drops of inocula per leaflet, depending on leaflet size. In collecting leaf disks for RNA-seq (see below), leaflets all had six drops of inocula. Mock-inoculated and *A. solani*-inoculated samples were from different leaves of the same plant in both mock- and thiamin-treated plants. Four biological replicates (one biological replicate = one plant) were used for each treatment, with a minimum of four leaves per plant. To provide moisture (relative humidity > 90%) and encourage disease development, a humidifier (AquaOasis, Johnston, IA, USA) was used for 2 h post-inoculation in the late afternoon (16:00) and subsequently for 2 h every morning and evening. A black shade tarp purchased from a local

retailer was draped over the chamber immediately post-inoculation in the late afternoon and removed the following morning.

Lesion measurements

Disease severity was determined after three to four days by modeling the lesions using the trace function and measuring the area of each lesion in mm^2 via ImageJ (Schneider et al. 2012). For the detached leaf assay, the value recorded for each plant is the mean lesion area across all 12 lesions. For the whole plant assay, all lesions on a single plant were measured via ImageJ and reported as a single mean lesion area per biological replicate (10 to 42 lesion area measurements per plant). For the systemic resistance assay, lesion diameter was recorded instead of area.

RNA extraction

Whole plants (n=3 for each treatment) were spray treated with 10 mM thiamin or mock solution. Four hours post treatment, three leaflets from the same leaf were inoculated with *A. solani*. Three biological replicate samples (leaves from one plant = one biological replicate) were collected from both *Alternaria*-inoculated and non-inoculated leaves on both thiamin-treated and mock-treated plants at three time points (12, 24, and 48 hours post-inoculation (hpi)). For each time point, treatment groups were assigned as follows: “mock_Alt_local”, mock-treated, *A. solani*-inoculated leaves; “mock_distal”, mock-treated, non-inoculated/distal leaves; “thi_Alt_local”, thiamin-treated, *A. solani*-inoculated leaves; and “thi_distal”, thiamin-treated, non-inoculated/distal leaves (Fig. S1). For samples from each time point and treatment group, an

8-mm diameter hole punch was used to take six leaf discs from the inoculated area, weighing a total of 50-60 mg per sample. Non-inoculated, mock- and thiamin-treated samples were collected concurrently with inoculated samples and were therefore assigned hpi designations of 12, 24, and 48 hpi accordingly. Samples were immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction. Frozen leaf tissue was first homogenized using a mortar and pestle in liquid nitrogen, and total RNAs were extracted using the PureLink™ RNA Mini Kit (Invitrogen, Waltham, MA, USA) using the manufacturer's instructions. Trace genomic DNA was removed via DNase I treatment via DNA-*free*™ Kit (Invitrogen). RNA was precipitated via one volume of 4 M lithium chloride (Millipore Sigma) incubated at 4°C overnight. After centrifugation at 13,250 g for 30 min at 4°C, the pellet was washed with 200 µL 70% ethanol and resuspended in RNase-free water. RNA integrity was evaluated via gel electrophoresis, and concentrations and potential contamination were assessed via a NanoDrop One^{C} spectrophotometer (ThermoScientific, Waltham, MA, USA), using absorbance ratios of A260/280 and A260/230 ≥ 2.0 for cut-off. RIN values were obtained via Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) and ranged in value from 6.6 to 9.

RNA-sequencing

Total RNA was sent to Novogene (Sacramento, CA, USA) for sequencing via Illumina platform. Messenger RNAs were first purified from total RNA using poly-T oligo-attached magnetic beads. Random hexamer primers were used for first strand cDNA synthesis followed by a second strand cDNA synthesis via Illumina NovaSeq platform. After end repair, A-tailing, adapter ligation, size selection, amplification, and purification, the 156-bp paired-end libraries

were sequenced using the Illumina NovaSeq 6000 Sequencing System (Illumina, San Diego, CA, USA). Adapters used for paired-end sequencing were as follows: 5' Adapter: 5'-AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTC-GGTGGTCGCCGTATCATT-3'; 3' Adapter: 5'-GATCGGAAGAGCACACGTCTGAAC-TCCAGTCACGGATGACTATCTCGTATGCCGTCTTCTGCTTG-3'.

RNA-seq data analysis

FASTQ file read quality was evaluated via FastQC (Andrews 2010), and adapters were trimmed via Trim Galore! (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) with default options, specific adaptor sequences, and filtering for Phred score $\geq$ 20 (Martin 2011). Genome indexing and mapping of trimmed, filtered reads to the potato reference genome DM_1-3_516_R44 v6.1 (http://spuddb.uga.edu/dm_v6_1_download.shtml) was performed via HISAT2 (https://daehwankimlab.github.io/hisat2/) with default options. Output SAM files were converted to BAM format and mapping quality was analyzed via Samtools (http://www.htslib.org/). The High Confidence Gene Model Set annotations file for DM v6.1, in GFF3 format, was converted to GTF format via gffread (http://ccb.jhu.edu/software/stringtie/gff.shtml). Reads were aligned to annotated gene models from the high-confidence gene model GTF via featureCounts (http://subread.sourceforge.net/) with the following options: -p; --countReadpairs; and –transcript.

The DESeq2 package (Love et al. 2014) in R (version 1.34.0) was used to generate lists of differentially expressed genes (DEGs) for each comparison group. Genes were differentially

expressed between groups only if adjusted $p \leq 0.05$ and $|\log_2(\text{fold-change})| \geq 2.0$. The comparison groups were assigned as follows: “response to Alt (local)”, mock_Alt_local vs. mock_distal; “response to Alt (thi)”, thi_Alt_local vs. thi_distal; “response to thi”, thi_distal vs. mock_distal; and “response to thi (Alt), thi_Alt_local vs. mock_Alt_local (Fig S1). Gene Ontology (GO) term assignments (DM_1-3_516_R44_potato.v6.1.working_models.go.txt.gz) for the annotated genes in the DM v6.1 genome were downloaded from http://spuddb.uga.edu. GO term enrichments for DEGs were identified via the clusterProfiler (https://github.com/YuLab-SMU/clusterProfiler) package in R version 4.1.2. “biological process”, “molecular function”, and “cellular component” terms were analyzed for each list of upregulated and downregulated DEGs, respectively, compiled from the three comparisons and time points and the “universe” option set to the population of genes with $\geq$ 1 read across all FASTQ files.

Metabolite analysis by GC-MS

Leaf metabolites collected at 1, 6, and 12 h after thiamin treatment were analyzed by GC-MS as previously described (Bvindi et al. 2023). The difference in sampling times between RNA-seq and metabolite analysis is explained in the text below. Briefly, full leaflets were selected from three plants (one plant = one biological replicate, each plant had every timepoints) sprayed with 10 mM thiamin or mock solution at 1, 6, and 12 hours post treatment (hpt) and frozen and ground to powder using mortar and pestle in liquid nitrogen. Three biological replicate samples per treatment group for each time point, each of approximately 50 mg mass, were added to 700 µL extraction solvent (water:methanol:chloroform (1:2.5:1)) with an internal standard (40 µg/ml ribitol). Samples were placed on ice for 5 minutes on a shaking platform rotating at 130 rpm, and

then centrifuged at 4℃ for 2 minutes at 21,000 x *g* to pellet cellular debris. The supernatant was transferred to a clean microcentrifuge tube and 280 µL of water was added to separate the aqueous phase from the organic phase. After a 2-minute centrifugation at 21,000 x *g*, the upper aqueous phase was collected and placed into a clean microcentrifuge tube. The samples were frozen at -80 ℃, then placed into a centrifugal vacuum concentrator and lyophilized to dryness overnight. Dried samples were stored at -80℃ until further analysis. A no tissue extraction control (i.e., reagent blank) was included to assess if detected peaks are plant tissue-specific. Dried samples were resuspended in 20 µL 30 mg/mL methoxyamine hydrochloride in pyridine and incubated at 37°C for 1.5 h, with vigorous shaking. Next, 40 µL of N-methyl-N-(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane was added and the samples were incubated at 37°C for 30 additional minutes with vigorous shaking. Metabolites were separated in an Agilent 7890B GC system (Agilent Technologies) and detected with an Agilent 5977B MSD in EI mode scanning from 50 m/z to 600 m/z. Mass spectrum analysis, component identification and peak area quantification were performed with AMDIS (Davies 1998). Fold-change and pathway enrichment analyses were performed via MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/MetaboAnalyst/), with independent comparisons between thiamin- and mock-treated samples at each time point. Metabolites with at least two-fold difference between treatment groups, and with raw p-value < 0.05 were selected for pathway enrichment analysis via hypergeometric test and the *Arabidopsis thaliana*-derived KEGG pathways. Pathway enrichment p-values were manually adjusted via the post-hoc Benjamini-Hochberg method to control the false discovery rate (Benjamini and Hochberg 2018).

Data reporting and statistical analyses

Statistical analysis of assays with multiple comparisons was performed via one-way ANOVA followed by a Tukey's HSD test for multiple comparisons. Assays incorporating single comparisons were analyzed via Student's *t*-test or Welch's *t*-test. RNA-seq and GO enrichment analyses incorporated the post-hoc Benjamini-Hochberg method to control the false discovery rate. Unless otherwise stated, statistical analyses and plots were produced using Microsoft Excel, Venny (Oliveros J 2007) and/or R version 4.1.2.

Results

Thiamin reduces early blight severity in a dose- and time-dependent fashion

To determine the optimal dosage of thiamin for foliar applications, we tested the effect of five different concentrations of thiamin against *A. solani* lesion size via a detached leaf assay. In two independent trials, leaflets from plants treated with 1, 5, 10, 25, and 50 mM thiamin and mock solution were removed and inoculated with *A. solani*. The only thiamin dose that significantly ($p < 0.05$) attenuated early blight lesion area in comparison to mock-treated leaves in both trials was 10 mM (32 and 52% decrease in lesion size in trials 1 and 2, respectively) (Table 1, Fig. S2). For this reason, a dosage of 10 mM was used for all subsequent experiments.

The durability of thiamin treatment in the reduction of *A. solani* lesion size was tested via a detached leaf assay in which *A. solani* inoculations were performed at 4 vs. 28 h post-thiamin treatment in two trials (Fig. 1A). While thiamin was effective in reducing lesion size when leaflets were inoculated with *A. solani* at 4 hpt, there was no observable effect at 28 hpt, indicating that

the effect of thiamin application is transitory. Consequently, all following thiamin treatments were performed at the optimal 10 mM concentration and with inoculations occurring at 4 hpt.

Utilizing the optimized thiamin dosage and timing, we surveyed the efficacy of thiamin in priming immunity against *A. solani* in whole plants. We observed that thiamin reduced the size of lesions in two additional independent trials by 34% and 32%, respectively (Fig. 1B).

To explore whether repeated thiamin applications desensitize potato to the corresponding immunity priming, we incorporated a detached leaf assay utilizing multiple applications of thiamin. Across the first and second applications, thiamin reduced lesion size by 29% and 22%, respectively, suggesting that repeated thiamin applications at the tested time points do not desensitize the plants to thiamin priming (Fig. 1C).

To evaluate the efficacy of thiamin as a priming agent for systemic immunity against *A. solani*, we designed a bagged leaf assay incorporating two cultivars of potato. We observed that unbagged, thiamin-treated leaves had reduced lesion size in comparison to bagged leaves (Fig 1D). Lesions of bagged, thiamin-treated leaves were similar to those of unbagged and bagged mock-treated leaves, suggesting that the thiamin treatments only locally reduce *A. solani* lesion development.

RNA-seq experimental design

To shed light on potential regulatory and response pathways of thiamin priming against *A. solani*, we utilized RNA-seq to investigate the transcriptome. Because we observed thiamin

only acts locally to attenuate early blight disease symptoms, the RNA-seq experiment was designed to identify genes specifically involved in local response to *A. solani* infection and thiamin treatments. Namely, locally inoculated *A. solani* leaves were compared against non-inoculated distal leaves from *A. solani*-inoculated plants to reduce the population of DEGs to only plant genes involved in local, but not systemic, response to *A. solani.* This design also replicates possible field conditions for early blight disease pressure, in which individual plants could possess a mix of both locally-infected and distal, non-infected foliage. Plants were separately treated with thiamin or mock solution, and at 4 hpt, leaves were inoculated with either *A. solani* ("local") or a mock solution ("distal"), after which leaf samples were collected 12, 24, and 48 hpi; these time points correspond to no visible lesion, beginning of lesion, and clear lesion, respectively. For each time point, samples were collected in four treatment groups: mock-treated and inoculated ("mock_Alt_local"); mock-treated and non-inoculated ("mock_distal"); thiamin-treated and inoculated ("thi_Alt_local"); and thiamin-treated and non-inoculated ("thi_distal") (Fig. S1).

Across all samples, 88.14% of trimmed reads mapped to the potato reference genome, and 86.67% of paired reads aligned to annotated gene models (Table S1). Principal Component Analysis was conducted on paired read counts aligned to genome features and revealed strong concordance among the biological replicates (Fig. S3). Differentially expressed genes (DEGs) were computed for four treatment comparisons (Fig. S1). To survey the local transcriptional response to *A. solani* ("response to Alt (local)"), mock-treated, locally inoculated ("mock_Alt_local") samples were compared to samples derived from mock-treated, distal/non-inoculated tissue ("mock_distal"). The response to *A. solani* was also surveyed in the context of thiamin-treated

plants ("response to Alt (thi)"), via comparison of "thi_Alt_local" to "thi_distal". Direct response to thiamin ("response to thi") was assessed using samples from distal/non-inoculated tissue: "thi_distal" vs. "mock_distal". The response to thiamin in the context of an *A. solani* infection ("response to thi (Alt)") was also surveyed in the background of locally inoculated tissue ("thi_Alt_local" vs. "mock_Alt_local"), however only 16 DEGs were identified at 12 hpi, 139 DEGs were identified at 24 hpi, and no DEGs were identified at 48 hpi, suggesting that transcriptional response to *A. solani* largely masks the transcriptional response to thiamin. Distributions of DEG expression fold-change and statistical significance by comparison group were visualized via volcano plots (Fig. S4).

Overall transcriptional responses to *A. solani* and thiamin

In the "response to thi" comparison (thiamin-treated vs. non-treated samples from distal/non-inoculated leaves), comparatively few DEGs were identified, including 111, 92, and 168 DEGs at 12, 24, and 48 hpi, respectively, with two DEGs shared across all timepoints (Fig. 2A; Tables S2-S4). The response to thiamin was also surveyed in the context of *A. solani* infection ("response to thi (Alt)"), yielding 16 and 139 DEGs at 12 and 24 hpi, respectively, all downregulated (Tables S5-S6). In "response to Alt (local)" (locally inoculated vs. distal/non-inoculated leaves; mock treatment), there were 2,421, 2,258, and 3,836 DEGs (Fig 2B; Tables S7-S9), compared to 1,217, 2,576, and 2,824 in "response to Alt (thi)" (locally inoculated vs. distal/non-inoculated leaves; thiamin treatment), at 12, 24, and 48 hpi, respectively (Fig 2C; Tables S10-S12). A total of 1,175 DEGs were common to all 3 timepoints in "response to Alt (local)", in comparison to 715 DEGs common to all time points in "response to Alt (thi)" (Fig. 2B-

C). In a comparison of the "response to Alt (local)" and "response to Alt (thi)" transcriptional responses across time points, the former exhibited a larger volume of DEGs at 12 and 48 hpi, with the inverse observed at 24 hpi (Fig. 2D-F). However, DEGs exhibited a large degree of overlap between both groups, with 954, 1,508, and 2,389 DEGs identified in both groups at 12, 24, and 48 hpi, respectively (Fig. 2D-F). Taken together, these results suggest a much more robust transcriptional response to *A. solani* infection than thiamin treatments and a shift in transcriptional response to *A. solani* in the context of thiamin pretreatment.

Thiamin treatment influences transcriptomic and metabolomic pathways in primary metabolism

To probe potential pathways through which thiamin primes immune response, using DEGs in the direct response to thiamin, absent pathogen ("response to thi"; Tables S2-S4), we performed GO enrichment analysis on upregulated and downregulated DEGs for each of the three time points. At 24 hpi, upregulation of two fatty acid desaturase genes contributed to significant enrichment for multiple fatty acid-associated GO terms, and upregulated glycosyl hydrolase/chitinase genes conferred enrichment for terms corresponding to chitinases (Fig. 3A; Tables S3, S13). At 48 hpi, enriched GO terms for upregulated DEGs corresponded to protease inhibitor and cytochrome P450 genes, as well as two peroxidases associated with GO terms for fatty acid alpha-oxidation and responses to ROS, SA, and nitric oxide (NO). Among all transcripts, Soltu.DM.02G034320, which encodes an ortholog to WRKY45, was the most upregulated DEG 12 hpi and 24 hpi and the third most upregulated DEG 48 hours hpi ($\log_2$FC ~ 20). For downregulated genes, significantly enriched GO terms were observed only at 12 hpi and were broadly associated

with photosynthesis GO terms due to a group of underlying genes encoding chlorophyll-binding proteins (Fig. 3B; Tables S2, S13). We also analyzed the transcriptomic response to thiamin in the context of infection ("response to thi (Alt)"), using leaf tissue locally infected with *A. solani*. A variety of significantly enriched GO terms were identified from upregulated DEGs at 12 hpi, with most corresponding to a single DEG (Fig. 4A; Tables S5, S14), putatively encoding an ATPase. Downregulated DEGs conferred significant enrichment of GO terms at 12 and 24 hpi, with a number of terms associated with the chloroplast, carotenoids, photosynthesis and Calvin cycle processes (Fig. 4B; Tables S5-S6, S14). These results suggest a comparatively limited transcriptional response to thiamin treatment that is at least partially masked/obscured by *A. solani* infection at the tested time points.

Because thiamin, in its pyrophosphorylated form, has been shown to increase the activities of the thiamin-dependent enzymes pyruvate dehydrogenase, α-ketoglutarate (α-KG) dehydrogenase, and transketolase in Arabidopsis (Bocobza et al. 2013), we hypothesized that thiamin priming of plant defense involves changes in thiamin-dependent metabolic pathways. Because the direct response to thiamin treatment in distal/non-inoculated leaves yielded a limited transcriptional response, and following our observation that thiamin primes immune response with a limited treatment window (Fig. 1A), we surmised that the metabolomic response to foliar thiamin treatment may evolve earlier than the time course utilized in our RNA-seq experimental design, with thiamin treatment evoking changes in metabolite content in advance of changes in gene expression. Accordingly, we utilized GC-MS to analyze metabolic changes in response to thiamin at earlier time points than those used for RNA-seq. Plants were treated solely

with 10 mM thiamin and surveyed for the abundance of polar metabolites, with sampling at 1, 6, and 12 hpt. A total of 92 analytes were identified in thiamin- and mock-treated samples (Table S17), and significant upregulation and downregulation of these compounds by thiamin was determined via comparison to mock-treated samples at each time point.

At 1 hpt, we identified eight significantly upregulated compounds (Fig. 5A), including D-erythrose-4-phosphate, a direct product of thiamin-dependent transketolase activity in the Calvin cycle and the non-oxidative branch of the pentose phosphate pathway (Sharkey 2021) (Fig. S5). At 1 hpt, we also detected in thiamin-treated samples significantly increased levels of glutamate, asparagine and putrescine, metabolites that are synthesized from the TCA cycle intermediates α-KG and oxaloacetate (Fig. S5). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis revealed that the eight analytes upregulated at 1 hpt contribute to enrichment in three metabolic pathways: “alanine, aspartate, and glutamate metabolism”, via accumulation of asparagine and glutamate; and “glutathione metabolism” and “arginine and proline metabolism”, via accumulation of glutamate and putrescine (Table S18). At 6 hpt, five and 25 compounds significantly accumulated or decreased, respectively (Fig. 5B). Compounds that decreased contributed to six distinct metabolic pathways, corresponding to metabolism of various amino acids, galactose, and glyoxylate and dicarboxylate, as well as the TCA cycle (Table S18, Fig. S5). At 12 hpt, only two upregulated compounds, 4-methyl-5-thiazoleethanol (sulfurol) and 3,5-dihydroxyphenylglycine, were identified (Fig. 5C), with no corresponding significant pathway enrichment. Notably, across all time points, 4-methyl-5-thiazoleethanol significantly accumulated as a result of thiamin treatment. 4-Methyl-5-

thiazoleethanol is a thiazole moiety of thiamin, suggesting that all or a portion of the applied thiamin was rapidly degraded. However, GC-MS analysis revealed that the thiamin product (≥98%) applied to leaves contained traces of 4-methyl-5-thiazoleethanol (data not shown), which could have contributed to the pool of 4-methyl-5-thiazoleethanol detected in leaf samples.

Transcriptional responses to *A. solani* infection

To validate the transcriptional response to *A. solani*, we performed GO enrichment analysis utilizing the DEGs identified in "response to Alt (local)" and "response to Alt (thi)" (Fig. 6A-B; Tables S15-S16). Upregulated DEGs in both treatment groups were found to be significantly enriched for GO terms such as aromatic amino acid, terpene, phenylpropanoid, lignin, and chorismate biosynthesis, fatty acid oxidation, and response to oxidative stress. At 12 hpi, the "response to jasmonic acid" term was significantly enriched only in "response to Alt (thi)", encompassing PR protein, lipoxygenase, JA-ZIM (JAZ) protein, ACC synthase, and ubiquitin ligase genes, as was the "response to fungus" term (Fig. 6A; Tables S10, S16). Additionally, genes corresponding to phenylalanine ammonia lyase (PAL) enzymes conferred significant enrichment of multiple PAL GO terms specifically in "response to Alt (thi)" at 12 hpi. At 48 hpi, "response to Alt (thi)" specifically exhibited significant enrichment for certain classes of GO terms, including: oxidative stress and lignin biosynthesis, via peroxidase genes; terpene biosynthesis; cell death, via phospholipase genes; and responses to chitin, via genes corresponding to U-box and LysM domain proteins.

Analysis of GO enrichment among downregulated DEGs in “response to Alt (local)” and “response to Alt (thi)” highlighted a variety of GO terms associated with photosynthesis, such as “light harvesting in PSI”, “response to light stimulus”, “chlorophyll binding” activity, and cellular component terms associated with localization to the chloroplast (Fig. 6B; Tables S15-S16). Underlying genes comprise a range of gene products, such as chlorophyll-binding proteins, components of photosystems I and II, and Calvin cycle enzyme classes such as ribulose bisphoshate carboxylases (RuBisCo) and glyceraldehyde 3-phosphate (G3P) dehydrogenases (Tables S7-S12, S15-S16). Broadly, a pattern was observed wherein “response to Alt (local)” downregulated DEGs were enriched for photosynthesis-related functions and components by 12 hpi. However, thiamin treatment conferred a possible delay in this effect, with “response to Alt (thi)” downregulated DEGs exhibiting similar enrichments starting at 24 hpi, instead (Fig. 6B). Additionally, “response to Alt (thi)” downregulated DEGs were significantly enriched for multiple GO terms related to primary metabolism. At 48 hpi, downregulation of glycolytic fructose 1,6-bisphosphate (FBP) aldolase and G3P dehydrogenase genes yielded significant enrichment for GO terms corresponding to reductive pentose phosphate cycle and FBP processes and activities (Fig. 6B; Tables S12, S16). Interestingly, such downregulation was not observed in “response to Alt (local)”, indicating a direct effect of thiamin treatment on primary metabolic pathways.

Discussion

In this study, we investigated thiamin, also known as vitamin B1, for its ability to induce immunity in potato to the fungal pathogen *A. solani*. We also sought to evaluate the potential

modes of action of thiamin as a priming agent in potato, incorporating surveys of transcriptomic and metabolic responses via RNA-seq and GC-MS, respectively.

Thiamin foliar application triggers a short, non-systemic resistance response

Utilizing detached leaf and whole plant assays, we observed that 10 mM was an optimal concentration for foliar applications of thiamin, yielding consistent reductions (32-52%) of foliar lesion area caused by the fungal pathogen *A. solani* (Table 1; Fig. 1B-C). This concentration is within the range of concentrations that have been reported in other phytopathosystems (Ahn et al. 2007; Boubakri et al. 2012). We found that the protection provided by thiamin treatments was short lived, wherein protection against *A. solani* was abated when inoculations were performed later than 4 hpt (Fig. 1A). These results contrast with a previous study in which thiamin conferred resistance to *Magnaporthe grisea* in rice and to *Pseudomonas syringae* in Arabidopsis for up to 15 and 5 days after application, respectively (Ahn et al. 2005; Ahn et al. 2007). This short-lived effect may be attributed to the rapid degradation of thiamin to its moieties, as indicated by the accumulation of 4-methyl-5-thiazoleethanol (sulfurol), the thiazole moiety of thiamin, shortly after foliar application (Fig. 5), which may occur more rapidly and quantitatively in potato than in other plant species, although further quantification is needed to assess the contribution of trace levels of sulfurol detected in the thiamin product applied to foliage. Additionally, we observed that reapplication of thiamin one-week post-initial treatment still conferred enhanced resistance (Fig. 1C), indicating that repeated thiamin application did not desensitize plants to priming.

We incorporated a bagged-leaf inoculation assay to evaluate the induction of systemic acquired resistance (SAR) by thiamin treatment and confirmed that thiamin immune priming does not act systemically (Fig. 1D). To our knowledge, only one previous study of Arabidopsis plants has reported a systemic induction of defense responses induced by thiamin treatment (Ahn et al. 2005). In this study, the authors applied thiamin spray to rosette leaves and collected cauline leaves, protected from thiamin spray with plastic, for quantification of the expression of the SAR marker gene *PR-1*. The expression of *PR-1* was induced in untreated cauline leaves, indicating a SAR response. However, the authors did not test whether untreated cauline leaves displayed increased resistance to pathogens. Our transcriptomics analysis indicated that thiamin directly induced the expression of two *PR* genes at 24 and 48 hpi in treated leaves (Tables S3-S4), similarly to *PR* genes upregulated in thiamin-treated leaves of rice (*PR-1*, *PR-10*, *PR-11*) and Arabidopsis (*PR-1*) (Ahn et al. 2005; Ahn et al. 2007). However, our RNA-seq experimental design did not facilitate analysis of expression of SAR markers in untreated distal leaves. Therefore, further evaluation of the apparent lack of induced resistance in distal tissues in potato will require additional experiments. In future studies incorporating other plant species, it will be important to assess the presence of SAR using both disease assays and defense marker genes.

Thiamin induces the expression of genes associated with the biosynthesis of secondary metabolites, redox biological processes, and fatty acid metabolism

We utilized RNA-seq and GO enrichment analysis to explore potential regulatory and response pathways activated by thiamin during immune priming. The results of these analyses revealed differential expression of genes associated with several distinct secondary metabolic pathways. First, amongst genes that were induced in response to thiamin treatment ("response to thi") (Fig. 3A; Tables S3-S4, S13), several belong to the phenylpropanoid metabolic pathway, including genes encoding PAL ($log_2FC = 3.31$, 24 hpi), ferulic acid 5-hydroxylase ($log_2FC = 2.28$ 12 hpi), and cinnamate 4-hydroxylase ($log_2FC = 2.51$ 48 hpi). Previous reports have shown that genes with predicted roles in phenylpropanoid biosynthesis were induced by thiamin in other plant species such as tobacco and grapevine (Ahn et al. 2005; Boubakri et al. 2013). Therefore, our results and previous reports convey a general induction of the phenylpropanoid pathway in response to thiamin across plant species and support a possible role of this pathway in thiamin priming. Second, in addition to phenylpropanoid pathway, a gene annotated as a cytochrome P450 superfamily protein was amongst the top upregulated genes ($log_2FC > 21.37$) in "response to thi" at 24 and 48 hpi (Tables S3-S4). A tBLASTn search of the NCBI core nucleotide database showed that its closest homolog in Arabidopsis encodes a flavonoid 3'-hydroxylase, which is involved in flavonoid biosynthesis (Schoenbohm et al. 2000), suggesting that flavonoid biosynthesis may also contribute to thiamin priming. Last, our RNA-seq results also showed

strong (up to $log_2FC = 21.37$) induction of genes annotated as terpene synthases at 12, 24, and 48 hpi. Terpenes are important secondary metabolites in plant defense against insects and fungal pathogens (Trapp and Croteau 2001).

GO enrichment analysis also revealed that terms associated with chitinases and protease inhibitors were enriched in the annotations of DGEs upregulated in “response to thi” (Fig. 3A). In grapevine, genes encoding basic class I chitinase, chitinase 4-C, and serine protease inhibitor proteins were also induced 24 to 48 hours following thiamin treatment with no pathogen (Boubakri et al. 2012), indicating that thiamin induces similar sets of genes associated with these terms across plant species. Other GO terms enriched in “response to thi” included redox biological processes, e.g., “cellular response to nitric oxide” and “cellular response to ROS”, due to the induced expression of peroxidases (Fig. 3A, Table S13). Peroxidases have diverse enzymatic functions within the cell, but are notably associated with the detoxification of ROS generated during pathogen defense signaling and the cell death response. An increase in total antioxidant potential was reported in response to thiamin treatment absent pathogen in grapevine (Boubakri et al. 2013). Furthermore, there was enrichment of fatty acid metabolism functions in non-inoculated, thiamin-treated (“response to thi”) leaves at 48 hpi due to the upregulation of peroxidase- and fatty acid desaturase-encoding genes (Fig. 3; Tables S2-S4, S13). Upregulated genes in these classes were also identified at 12 and 24 hpi. Fatty acids are precursors for the phytohormone jasmonic acid (JA) (51, 52). Notably, in “response to thi”, we observed upregulation of candidate JA biosynthesis genes encoding lipoxygenases (12 and 48 hpi) and an allene oxide synthase (48 hpi) (Tables S2, S4).

Thiamin priming in potato likely involves SA and WRKY45

The most differentially regulated gene in "response to thi" at 12 and 24 hpi, and amongst the top three at 48 hpi, encodes a putative WRKY DNA-binding protein; this protein is annotated as a probable WRKY45 in NCBI. In rice, WRKY45 is transcriptionally upregulated by the chemical inducers benzothiadiazole and SA, and WRKY45-knockdown rice plants were compromised in their ability to induce resistance to rice blast by benzothiadiazole (Shimono et al. 2007). Priming of plant defenses by both thiamin and benzothiadiazole has been shown to depend on the SA signaling pathway (Ahn et al. 2007; Shimono et al. 2007), and correspondingly, the GO term "cellular response to salicylic acid stimulus" was enriched among genes upregulated at 48 hpi in "response to thi" (Fig. 3A). More recently, WRKY45 was shown to positively regulate age-triggered and dark-induced leaf senescence in Arabidopsis (Barros et al. 2022; Chen et al. 2017). Leaf senescence is a programmed cell death process, which is characterized by the degradation of chloroplasts and the decrease in cellular metabolic activity. Interestingly, GO enrichment analysis of downregulated DEGs indicated a broad reduction in expression of photosynthesis genes, such as chlorophyll-binding protein, photosystem (PS) component, and Calvin cycle enzyme genes, at 12 hpi (Fig. 3B; Tables S2, S13). Altogether, these results suggest that thiamin priming involves the SA signaling pathway, the induced expression of WRKY45, and eventually, the premature activation of leaf senescence.

Thiamin mimics the recognition of *A. solani* and boosts defense responses

In response to *A. solani* infection, we found that thiamin treatment ("response to Alt (thi)") enhanced transcription of a variety of gene classes associated with pathogen defense in comparison to mock-treated plants ("response to Alt (local)"), as indicated by enrichment for GO terms corresponding to shikimate biosynthesis and PAL activity (Fig. 6; S15-S16). These terms include genes encoding proteins of various functions, such as peroxidases, PR proteins, U-box proteins, JAZ proteins, and chitinases, findings that are indicative of enhanced immune response (Tables S7-S12). Transcription of SA pathway, PR, and chitinase genes is well characterized in the context of plant-pathogen interactions, and a recent study reported activation of these gene classes, as well as the requirement of SA signaling, particularly in response to *A. solani* infections of potato (Brouwer et al. 2020). Interestingly, 308 of 329, or 94% of genes differentially expressed in the response to thiamin absent pathogen challenge ("response to thi") across the three time points were amongst the population of DEGs identified in "response to Alt (local)" (Tables S2-S4, S7-S9). Even more remarkably, genes that were the most upregulated (i.e., $\log_2$FC > 20) in "response to thi" at 12, 24 and 48 hours after inoculation were exactly the same genes that were the most upregulated in "response to Alt (local)", e.g., WRKY45. These results indicate that thiamin mimics the recognition of *A. solani* by the plant and induces a core subset of genes to prime plants against *A. solani*.

Further analysis of the "response to Alt (thi)" and "response to Alt (local)" provides additional hypotheses about how thiamin protects against *A. solani*. Enrichment of a GO term for metabolism of shikimate, a precursor of chorismate and substrate for SA and phenylalanine

biosynthesis, was identified uniquely in “response to Alt (thi)” at 12 hpi (i.e., without corresponding enrichment in “response to Alt (local)”). It is noteworthy that erythrose-4-phosphate, a precursor of shikimate produced by thiamin-dependent transketolases, accumulated 1 h post-thiamin treatment, absent pathogen challenge (Fig. 5A), which may contribute to higher shikimate production. Furthermore, we identified specific enrichment for the “response to jasmonic acid” GO term encompassing PR protein, lipoxygenase, JAZ protein, ACC synthase, and ubiquitin ligase genes in “response to Alt (thi)” at 12 hpi (Fig. 6A; Table S16). Together, these findings convey that thiamin treatment may provide a boost towards defense responses in the first hours after inoculation. Additional experiments, including the measurement of enzymatic activities and additional metabolites, will be necessary to test this hypothesis.

Thiamin attenuates the expression of photosynthesis- and primary metabolism-related genes

One of the most apparent consequences of thiamin treatment, in the context of infection with *A. solani*, is the temporal shift in the attenuation of photosynthesis-related gene expression. Photosynthesis metabolism can be highly modified by the immune response in plants, wherein crosstalk with defense phytohormones and ROS produces a shift in metabolic resources to mount a defense against pathogens (Yang and Luo 2021; Zhu et al. 2015). While GO enrichment analysis of downregulated DEGs indicated a broad reduction in expression of photosynthesis genes, such as chlorophyll-binding protein, photosystem (PS) component, and Calvin cycle enzyme genes, at

12 hpi in “response to Alt (local)”, this effect was delayed in “response to Alt (thi)” leaves, with a similar pathway enrichment emerging instead at 24 hpi instead (Fig 6B; Tables S15-S16). In the *A. solani*-inoculated background (“response to thi (Alt)”), downregulation of chlorophyll and PS component and RuBisCo genes conferred GO term enrichments at 24 hpi as well (Fig. 4B; Tables S6, S14). Important defense phytohormones and signaling molecules like JA and nitric oxide are partially synthesized in the chloroplasts, and upon infection, transcriptional reprogramming is essential to produce these pro-defense molecules (Jasid et al. 2006; Serrano et al. 2016). Biosynthesis of fatty acids and aromatic amino acids is also compartmentalized to chloroplasts (Kim 2020), and per our observations, expression of genes associated with metabolism of fatty acids and derivatives of aromatic amino acids is activated by pathogen infection and further enhanced by thiamin treatment. Accordingly, thiamin treatment is likely attenuating photosynthesis prior to infection with *A. solani*, priming immunity, and/or enhancing immune response post-infection, possibly with an accompanying shift from photosynthesis to lipid and aromatic amino acid metabolism in the chloroplast.

By analysis of “response to Alt (thi)” samples, we also observed that thiamin suppressed the expression of genes involved in primary metabolism, including G3P dehydrogenases and FBP aldolases, at 48 h-post *A. solani* infection (Fig. 6B; Tables S12, S16). Primary metabolic pathways such as glycolysis, the TCA cycle, and the pentose phosphate cycle are closely intertwined with photosynthesis. Interestingly, three intermediates of the citric acid cycle, i.e., α-KG, fumarate and succinate, were depleted at 6 hpt compared to the control, suggesting an overall decrease in the flux of pyruvate towards the TCA cycle. Therefore, we hypothesize that the priming of immune

activity by thiamin may also result from a perturbation of primary metabolism that consequently shifts resources to activate pathogen defense pathways. Accordingly, future research should investigate thiamin-induced metabolic reprogramming in greater detail, focusing on the metabolite flux among the interconnected metabolic pathways that incorporate thiamin-dependent enzyme activities, with the goal of characterizing the resulting impacts to primary metabolism that inform the activation of immune priming by exogenous applications of thiamin.

Our findings demonstrate that foliar applications of thiamin decrease the size of lesions caused by the necrotrophic fungal pathogen *A. solani* in potato. However, immune priming was limited both temporally and to locally infected tissue, thereby presenting a challenge to immediate use in managing early blight disease in potato. Future experiments could investigate pathways to enhance potato immune response of potato to thiamin, such as combinations of thiamin with other chemical elicitors of pathogen defense with different modes of action. Transcriptomics analysis showed that the response of potato to thiamin involved expression of a gene set that largely overlaps with the transcriptomic response to *A. solani*, indicating that thiamin may elicit conditions that mimic pathogen challenge. Metabolite analyses indicated that thiamin triggered the depletion of several organic acids, which are typically depleted under low energy cellular status. We hypothesize that thiamin's mode of action is through perturbation of primary metabolism leading to induction the SA and JA signaling pathways.

Electronic supplementary material

Table S1. Quality control metrics for RNA sequencing data. Processed reads were mapped to the DM 1-3 516 R44 v6.1 reference genome. "hpi", hours post-inoculation. 1FASTQ files from Illumina paired-end sequencing were trimmed of adapter sequence via Trim Galore. 2Read pairs were mapped to the reference genome via HISAT2 and 3aligned to annotated, representative gene models via "featureCounts."

Table S2. Differentially expressed genes (DEGs) in "response to thi", 12 hours post-inoculation (hpi). DEGs were selected on the basis of adjusted p-value (APV) < 0.05 and |log2(fold-change)| (log2FC) > 2.

Table S3. Differentially expressed genes (DEGs) in "response to thi", 24 hours post-inoculation (hpi). DEGs were selected on the basis of adjusted p-value (APV) < 0.05 and |log2(fold-change)| (log2FC) > 2.

Table S4. Differentially expressed genes (DEGs) in "response to thi", 48 hours post-inoculation (hpi). DEGs were selected on the basis of adjusted p-value (APV) < 0.05 and |log2(fold-change)| (log2FC) > 2.

Table S5. Differentially expressed genes (DEGs) in "response to thi (Alt)", 12 hours post-inoculation (hpi). DEGs were selected on the basis of adjusted p-value (APV) < 0.05 and |log2(fold-change)| (log2FC) > 2.

Table S6. Differentially expressed genes (DEGs) in "response to thi (Alt)", 24 hours post-inoculation (hpi). DEGs were selected on the basis of adjusted p-value (APV) < 0.05 and |log2(fold-change)| (log2FC) > 2.

Table S7. Differentially expressed genes (DEGs) in "response to Alt (local)", 12 hours post-inoculation (hpi). DEGs were selected on the basis of adjusted p-value (APV) < 0.05 and |log2(fold-change)| (log2FC) > 2.

Table S8. Differentially expressed genes (DEGs) in "response to Alt (local)", 24 hours post-inoculation (hpi). DEGs were selected on the basis of adjusted p-value (APV) < 0.05 and |log2(fold-change)| (log2FC) > 2.

Table S9. Differentially expressed genes (DEGs) in "response to Alt (local)", 48 hours post-inoculation (hpi). DEGs were selected on the basis of adjusted p-value (APV) < 0.05 and |log2(fold-change)| (log2FC) > 2.

Table S10. Differentially expressed genes (DEGs) in "response to Alt (thi)", 12 hours post-inoculation (hpi). DEGs were selected on the basis of adjusted p-value (APV) < 0.05 and |log2(fold-change)| (log2FC) > 2.

Table S11. Differentially expressed genes (DEGs) in "response to Alt (thi)", 24 hours post-inoculation (hpi). DEGs were selected on the basis of adjusted p-value (APV) < 0.05 and |log2(fold-change)| (log2FC) > 2.

Table S12. Differentially expressed genes (DEGs) in "response to Alt (thi)", 48 hours post-inoculation (hpi). DEGs were selected on the basis of adjusted p-value (APV) < 0.05 and |log2(fold-change)| (log2FC) > 2.

Table S13. Enriched Gene Ontology (GO) terms identified in differentially expressed genes (DEGs) in "response to thi". DEGs (|log2(fold-change)| > 2.0) were separated into upregulated ("UP") and downregulated ("DOWN") "DE" groups and analyzed for enrichment (adjusted p-value ("APV") < 0.05) of Biological Process ("BP"), Cellular Component ("CC"), and Molecular Function

("MF") GO terms via clusterProfiler. Fold-enrichment ("FE") is the ratio of "gene ratio" to "background ratio" as reported by clusterProfiler. "tAPV", -log10-transformed tAPV.

Table S14. Enriched Gene Ontology (GO) terms identified in differentially expressed genes (DEGs) in "response to thi (Alt)". DEGs (|log2(fold-change)| > 2.0) were separated into upregulated ("UP") and downregulated ("DOWN") "DE" groups and analyzed for enrichment (adjusted p-value ("APV") < 0.05) of Biological Process ("BP"), Cellular Component ("CC"), and Molecular Function ("MF") GO terms via clusterProfiler. Fold-enrichment ("FE") is the ratio of "gene ratio" to "background ratio" as reported by clusterProfiler. "tAPV", -log10-transformed tAPV.

Table S15. Enriched Gene Ontology (GO) terms identified in differentially expressed genes (DEGs) in "response to Alt (local)". DEGs were separated into upregulated ("UP") and downregulated ("DOWN") "DE" groups and analyzed for enrichment (adjusted p-value ("APV") < 0.05) of Biological Process ("BP"), Cellular Component ("CC"), and Molecular Function ("MF") GO terms via clusterProfiler. Fold-enrichment ("FE") is the ratio of "gene ratio" to "background ratio" as reported by clusterProfiler. "tAPV", -log10-transformed tAPV.

Table S16. Enriched GO terms identified in DEGs in "response to Alt (thi)". DEGs (|log2(fold-change)| > 2.0) were separated into upregulated ("UP") and downregulated ("DOWN") "DE" groups and analyzed for enrichment (adjusted p-value ("APV") < 0.05) of Biological Process ("BP"), Cellular Component ("CC"), and Molecular Function ("MF") GO terms via clusterProfiler. Fold-enrichment ("FE") is the ratio of "gene ratio" to "background ratio" as reported by clusterProfiler. "tAPV", -log10-transformed tAPV.

Table S17. Metabolite concentrations in leaf samples in thiamin- or mock-treated plants at 1, 6 and 12 hpt as determined by GC-MS. Data were normalized by sample weight and using the

internal standard ribitol. M = mock-treated; T = thiamin-treated. 3,5-DHPG = 3,5-dihydroxyphenylglycine; sulfurol = 4-methyl-5-thiazoleethanol; D-erythrose-4-P = D-erythrose-4-phosphate; beta-1GalMe = methyl-beta-D-galactopyranoside; phenylglucoside = phenyl-beta-glucopyranoside; sinapic acid = 3,5-dimethoxy-4-hydroxycinnamate; methylgalactoside = methyl-beta-D-galactopyranoside; phenylglucoside = phenyl-beta-glucopyranoside; 6-OH-nicotinate = 6-hydroxynicotinate; D-GlcNAc = N-acetyl-D-glucosamine; alpha-KG = α-ketoglutarate.

Table S18. Pathway enrichment of metabolomics data following thiamin treatment. Via MetaboAnalyst 6.0, thiamin-treated samples were compared to mock-treated samples at 1, 6, and 12 hours post-treatment (hpt), and metabolites were selected on the basis of p-value < 0.05 and |log2(fold-change)| (log2FC) > 2. Selected metabolites were assigned to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, followed by hypergeometric testing for upregulated "UP" and downregulated "DOWN" pathways with post-hoc Benjamini-Hochberg (B-H) p-value correction.

Figure S1. Schema for treatment groups (left) and comparison groups (right) for RNA-seq differential expression analysis.

Figure S2. Representative pictures of lesions 3 days after inoculation with *A. solani* in plants treated with 10 mM thiamin or a mock solution.

Figure S3. Principal Component Analysis (PCA) plot of RNA-seq samples. Replicates from the same treatments are circled. Red circles indicate *A. solani*-inoculated samples while green circles indicate non-inoculated samples.

Figure S4. Volcano plots for differentially expressed genes (DEGs) at 12 (left), 24 (center), and 48 (right) hours post-inoculation. (A), “response to Alt (local).” (B), “response to Alt (thi).” (C), “response to thi (Alt).” (D), “response to thi.” “APV”, adjusted p-value.

Figure S5. Simplified schema of the Calvin cycle, glycolysis, the TCA cycle, and α-ketoacids catabolism with thiamin-dependent enzymatic steps. In orange squares are thiamin-dependent enzymes. TK, transketolase; PDH, pyruvate dehydrogenase; KGDH, 2-oxoglutarate (α-KG) dehydrogenase; BCKDH, branched-chain amino acids ketodehydrogenase. (A) Thiamin-dependent pathways where metabolites accumulated at 1 hpt with thiamin (red text). (B) Thiamin-dependent pathways where metabolites decreased at 6 hpt with thiamin (blue text).

Availability of data and materials

Raw Illumina sequencing reads are deposited at the NCBI Sequence Read Archive under the BioProject accession number PRJNA1149021.

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Table 1. Average lesion area of *A. solani* on plants treated with increasing concentrations of thiamin in two independent trials. Data are means ± S.D. of four biological replicates. Identical letters indicate no statistical difference between treatments as determined by ANOVA and Tukey's test. Spore concentrations were $2.1x10^4$ and $2.7x10^4$ spores per mL in trial 1 and 2, respectively.

Thiamin concentration (mM)	**Lesion size (mean ± SD)**	
	Trial 1	Trial 2
0	41.4 ± 8.2 [a]	52.6 ± 16.6 [a]
1	31.9 ± 4.4 [ab]	34.2 ± 17.0 [ab]
5	29.3 ± 4.5 [ab]	34.6 ± 8.9 [ab]
10	28.3 ± 4.0 [b]	25.2 ± 1.6 [b]
25	30.6 ± 6.2 [ab]	28.7 ± 5.5 [ab]
50	31.4 ± 3.8 [ab]	30.1 ± 5.4 [ab]

Figure legends

Fig 1. Effects of thiamin foliar application on lesions caused by *A. solani*. (**A**) Average lesion area of *A. solani* on plants treated with either 10 mM thiamin or a mock solution and inoculated either 4 or 28 h after treatment in two independent trials. Spore concentrations (spores per mL) were $2.1x10^4$ (4 h) and $2.5x10^4$ (24 h) for trial 1, and $1.7x10^4$ (4 h) and $1.8x10^4$ (24 h) for trial 2. (**B**) Average lesion area of *A. solani* on whole plants treated with either 10 mM thiamin or mock solution and inoculated 4 hr after treatment in two independent trials. Spore concentrations (spores per mL) were $1.5x10^4$ and $1.8x10^4$ for trial 1 and trial 2, respectively. (**C**) Average lesion area of *A. solani* in plants treated with one or two thiamin foliar applications. The second thiamin application was done seven days after the first one on the same plants. Spore concentrations (spores per mL) were $1.5x10^4$ and $2.1x10^4$ for the first and second inoculation, respectively. (**D**) Average lesion area of *A. solani* on bagged and unbagged leaves from plants treated with thiamin or a mock solution as determined by detached leaf assay. Spore concentration (spores per mL) was $5.8x10^3$. Treatments: MB = Bagged leaves from mock plants, MU = Unbagged leaves from mock plants, TB = Bagged leaves from thiamin-treated plants, TU = Unbagged leaves from thiamin-treated plants. Varieties: RB = Russet Burbank, RN = Russet Norkotah. All data are means ± S.E. of four biological repetitions, except (**D**) which had three and six biological replicates for mock- and thiamin-treated plants, respectively. Asterisks indicate a p-value of ≤ 0.05 (*) or ≤ 0.01 (**) when compared to mock treatment as determined by Student t-test, except for (**D**) where Welch's T test was used for comparing MU vs. TU and MU vs. TB.

Fig 2. Comparison of differentially expressed genes (DEGs) between treatment groups and timepoints. (**A**) Number of DEGs in "response to thi" (thi_distal vs. mock_distal) at 12, 24, and 48 hpi. (**B**) Number of DEGs in "response to Alt (local)" (mock_Alt_local vs. mock_distal) at 12, 24, and 48 hpi. (**C**) Number of DEGs in "response to Alt (thi)" (thi_Alt_local vs. thi_distal) at 12, 24, and 48 hpi. (**D-F**) Comparisons of DEGs between mock- and thiamin-treated plants in response to *A. solani* ("response to Alt (local)" and "response to Alt (thi)") at 12 (**D**), 24 (**E**), and 48 (**F**) hpi.

Fig 3. GO term enrichment of upregulated (A) and downregulated (B) DEGs in "response to thi". DEGs were selected on the basis of $|\log_2(\text{fold-change})| \geq 2$ and adjusted p-value < 0.05. Enriched GO terms were selected on the basis of adjusted p-value (APV) < 0.05. [column rows]. "hpi", hours post-inoculation. "tAPV", $-\log_{10}$-transformed APV. "FE", fold-enrichment. Blue-shaded terms, "Biological Process"; orange-shaded terms, "Cellular Component"; green-shaded terms, "Molecular Function".

Fig 4. GO term enrichment of upregulated (A) and downregulated (B) DEGs in "response to thi (Alt)". DEGs were selected on the basis of |log2(fold-change)| > 2 and adjusted p-value < 0.05. Enriched GO terms were selected on the basis of adjusted p-value (APV) < 0.05. "hpi", hours post-inoculation. "tAPV", -log10-transformed APV. "FE", fold-enrichment. Blue-shaded terms, "Biological Process"; orange-shaded terms, "Cellular Component"; green-shaded terms, "Molecular Function".

Fig 5. Volcano plots of metabolite concentrations in leaves of thiamin-treated versus mock-treated plants at 1 (A), 6 (B) and 12 (C) hpt. "FC", fold change.

Fig 6. GO term enrichment of upregulated (A) and downregulated (B) DEGs in “response to Alt (local)” (m_A) and “response to Alt (thi)” (t_A). DEGs were selected on the basis of $|\log_2(\text{fold-change})| \geq 2$ and adjusted p-value < 0.05. Enriched GO terms were selected on the basis of adjusted p-value (APV) < 0.05. “hpi”, hours post-inoculation. “tAPV”, $-\log_{10}$-transformed APV. “FE”, fold-enrichment. Blue-shaded terms, “Biological Process”; orange-shaded terms, “Cellular Component”; green-shaded terms, “Molecular Function”.

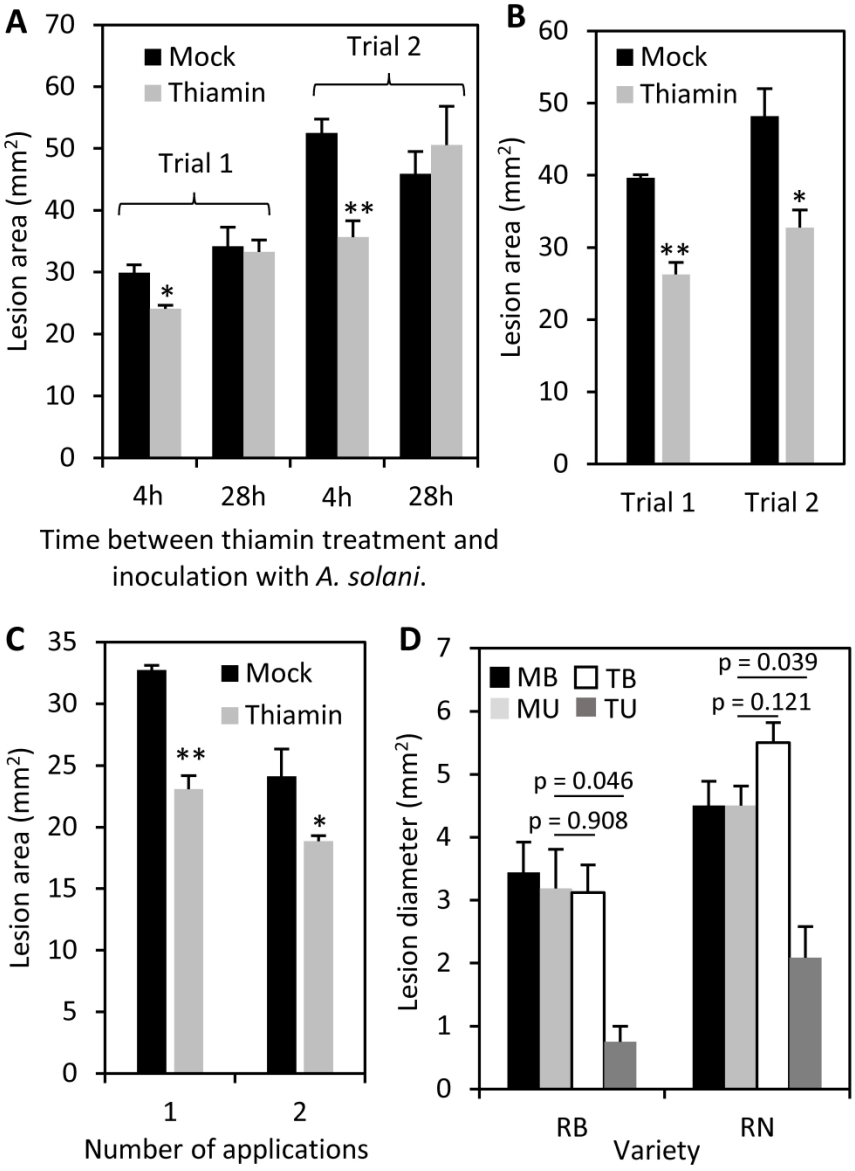

Fig 1. Effects of thiamin foliar application on lesions caused by A. solani. (A) Average lesion area of A. solani on plants treated with either 10 mM thiamin or a mock solution and inoculated either 4 or 28 h after treatment in two independent trials. Spore concentrations (spores per mL) were 21,563 (4 h) and 24,688 (24 h) for trial 1, and 16,785 (4 h) and 18,125 (24 h) for trial 2. (B) Average lesion area of A. solani on whole plants treated with either 10 mM thiamin or mock solution and inoculated 4 hr after treatment in two independent trials. Spore concentrations (spores per mL) were 15,469 and 17,813 for trial 1 and trial 2, respectively. (C) Average lesion area of A. solani in plants treated with one or two thiamin foliar applications. The second thiamin application was done seven days after the first one on the same plants. Spore concentrations (spores per mL) were 15,468 and 21,562 for trial 1 and trial 2, respectively. (D) Average lesion area of A. solani on bagged and unbagged leaves from plants treated with thiamin or a mock solution as determined by detached leaf assay. Spore concentration (spores per mL) was 5,875. Treatments: MB = Bagged leaves from mock plants, MU = Unbagged leaves from mock plants, TB = Bagged leaves from thiamin-treated plants, TU = Unbagged leaves from thiamin-treated plants. Varieties: RB = Russet Burbank, RN = Russet Norkotah. All data are means ± S.E. of four biological repetitions, except (D)

which had three and six biological replicates for mock- and thiamin-treated plants, respectively. Asterisks indicate a p-value of ≤ 0.05 (*) or ≤ 0.01 (**) when compared to mock treatment as determined by Student t-test, except for (D) where Welch's T test was used for comparing MU vs. TU and MU vs. TB.

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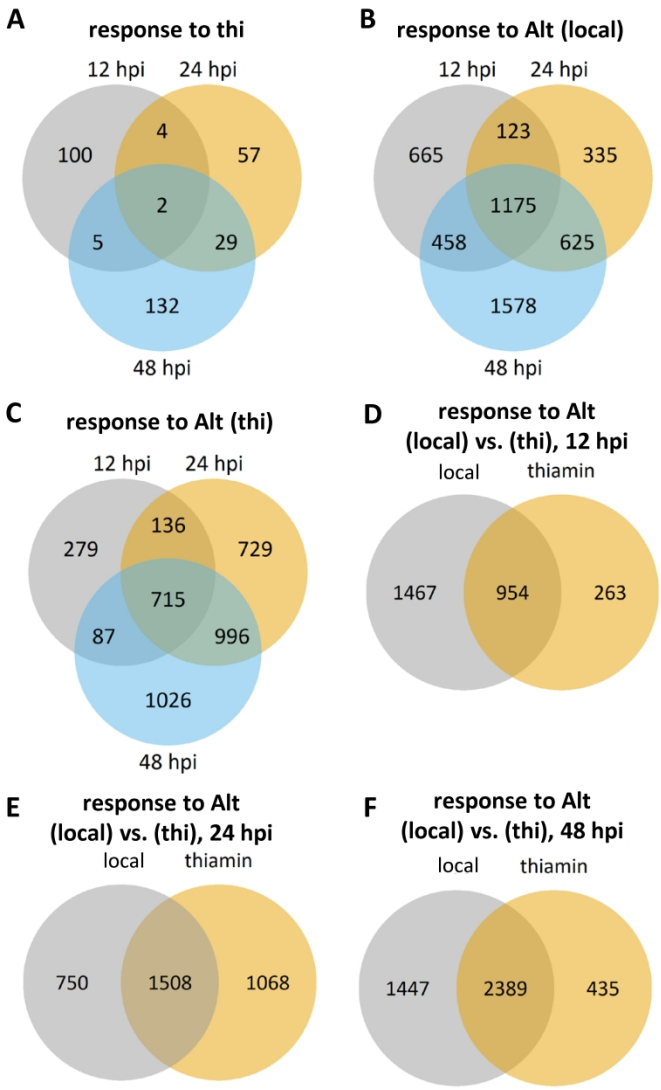

Fig 2. Comparison of differentially expressed genes (DEGs) between treatment groups and timepoints. (A) Number of DEGs in "response to thi" (thi_distal vs. mock_distal) at 12, 24, and 48 hpi. (B) Number of DEGs in "response to Alt (local)" (mock_Alt_local vs. mock_distal) at 12, 24, and 48 hpi. (C) Number of DEGs in "response to Alt (thi)" (thi_Alt_local vs. thi_distal) at 12, 24, and 48 hpi. (D-F) Comparisons of DEGs between mock- and thiamin-treated plants in response to A. solani ("response to Alt (local)" and "response to Alt (thi)") at 12 (D), 24 (E), and 48 (F) hpi.

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Fig 3. GO term enrichment of upregulated (A) and downregulated (B) DEGs in "response to thi". DEGs were selected on the basis of |log2(fold-change)| > 2 and adjusted p-value < 0.05. Enriched GO terms were selected on the basis of adjusted p-value (APV) < 0.05. [column rows]. "hpi", hours post-inoculation. "tAPV", -log10-transformed APV. "FE", fold-enrichment. Blue-shaded terms, "Biological Process"; orange-shaded terms, "Cellular Component"; green-shaded terms, "Molecular Function".

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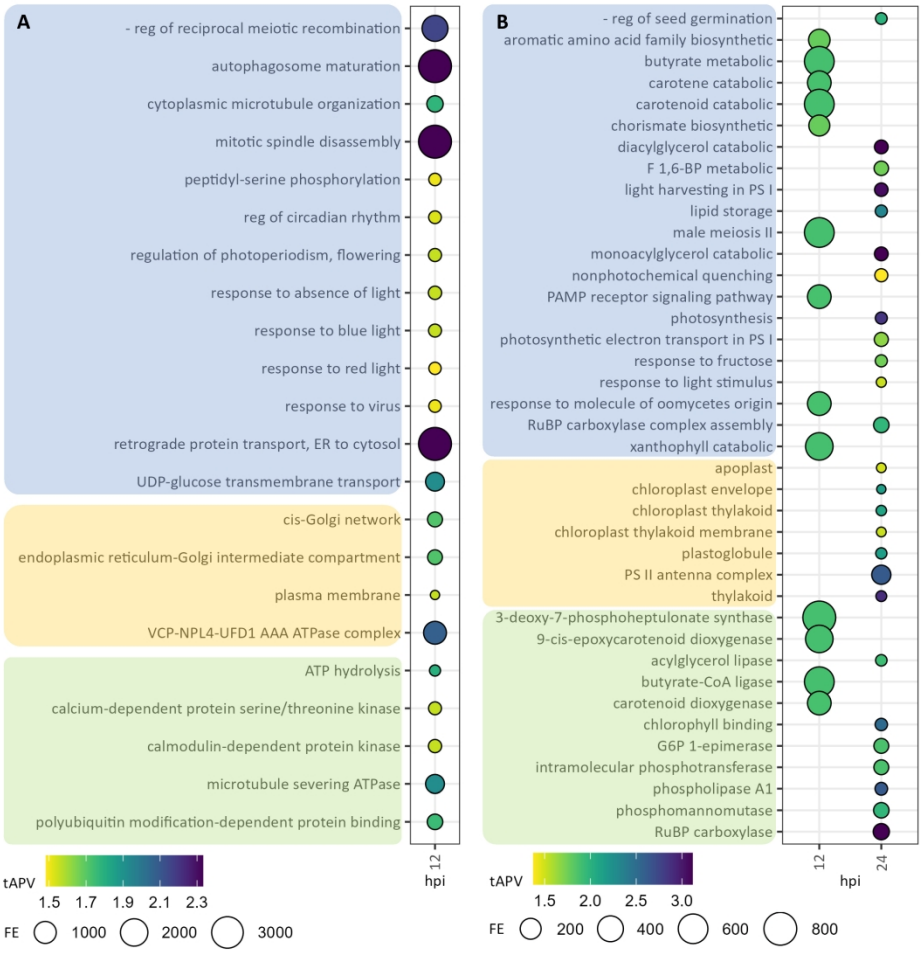

Fig 4. GO term enrichment of upregulated (A) and downregulated (B) DEGs in "response to thi (Alt)". DEGs were selected on the basis of |log2(fold-change)| > 2 and adjusted p-value < 0.05. Enriched GO terms were selected on the basis of adjusted p-value (APV) < 0.05. "hpi", hours post-inoculation. "tAPV", -log10-transformed APV. "FE", fold-enrichment. Blue-shaded terms, "Biological Process"; orange-shaded terms, "Cellular Component"; green-shaded terms, "Molecular Function".

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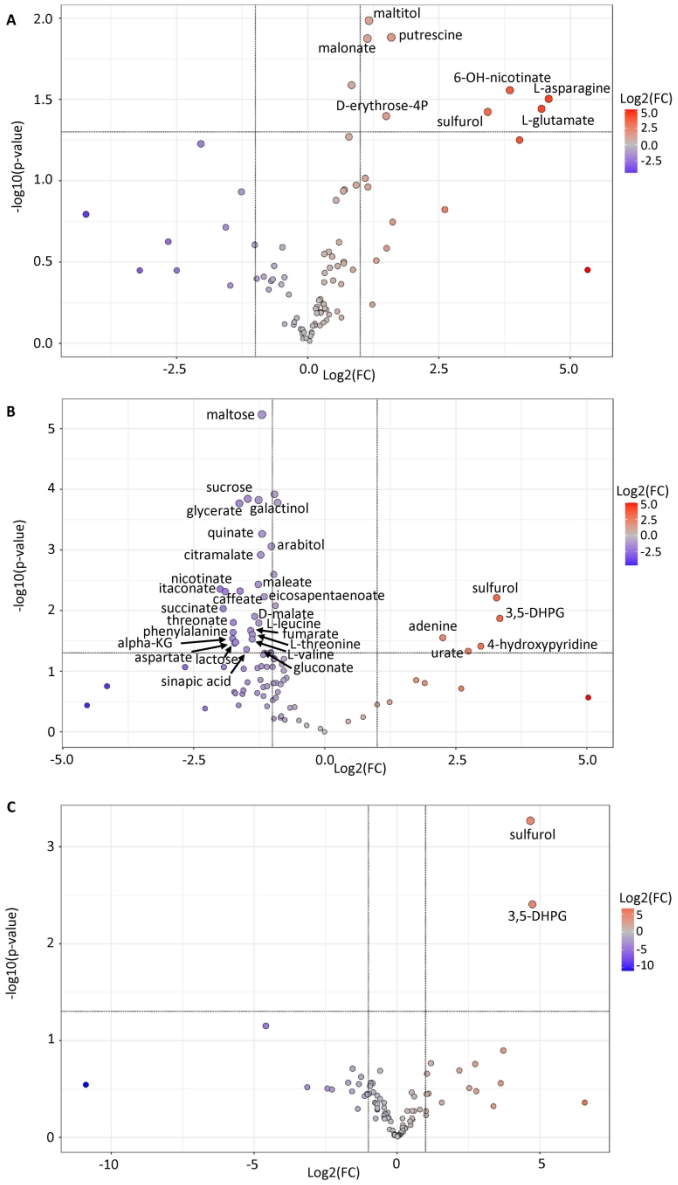

Fig 5. Volcano plots of metabolite concentrations in leaves of thiamin-treated versus mock-treated plants at 1 (A), 6 (B) and 12 (C) hpt. "FC", fold change.

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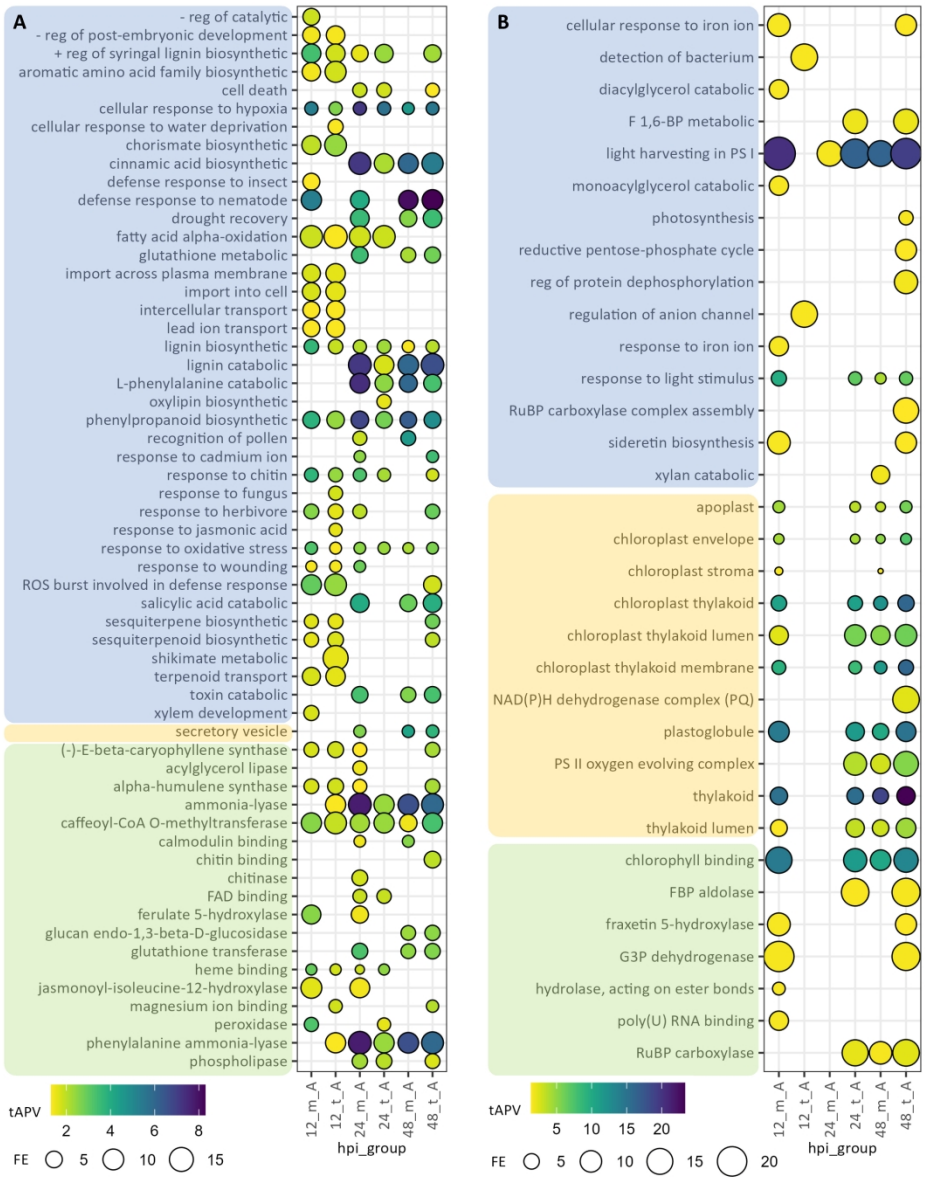

Fig 6. GO term enrichment of upregulated (A) and downregulated (B) DEGs in "response to Alt (local)" (m_A) and "response to Alt (thi)" (t_A). DEGs were selected on the basis of |log2(fold-change)| > 2 and adjusted p-value < 0.05. Enriched GO terms were selected on the basis of adjusted p-value (APV) < 0.05. "hpi", hours post-inoculation. "tAPV", -log10-transformed APV. "FE", fold-enrichment. Blue-shaded terms, "Biological Process"; orange-shaded terms, "Cellular Component"; green-shaded terms, "Molecular Function".

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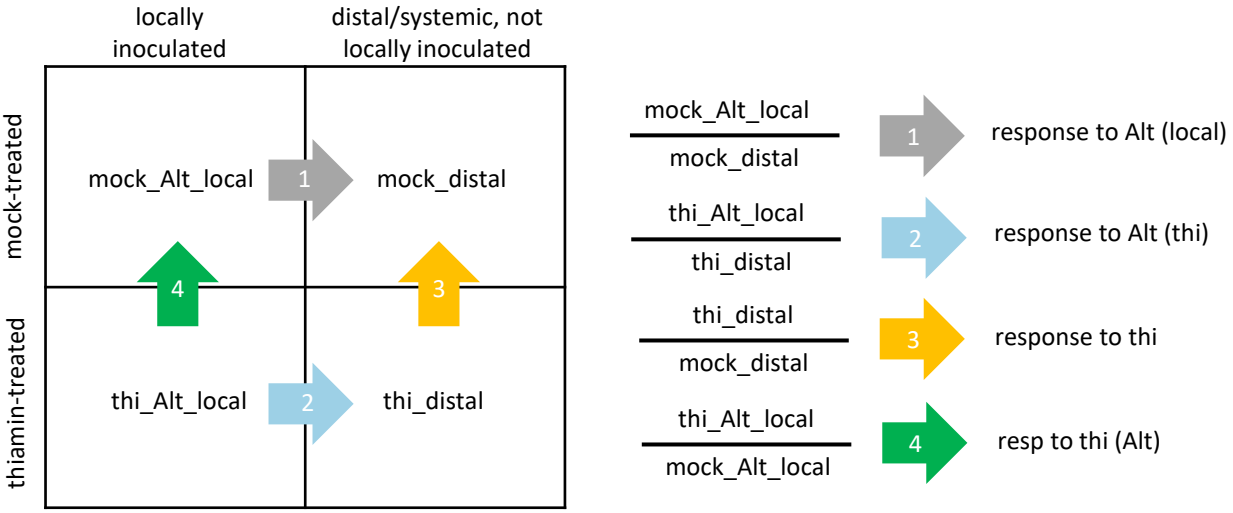

Figure S1. Schema for treatment groups (left) and comparison groups (right) for RNA-seq differential expression analysis.

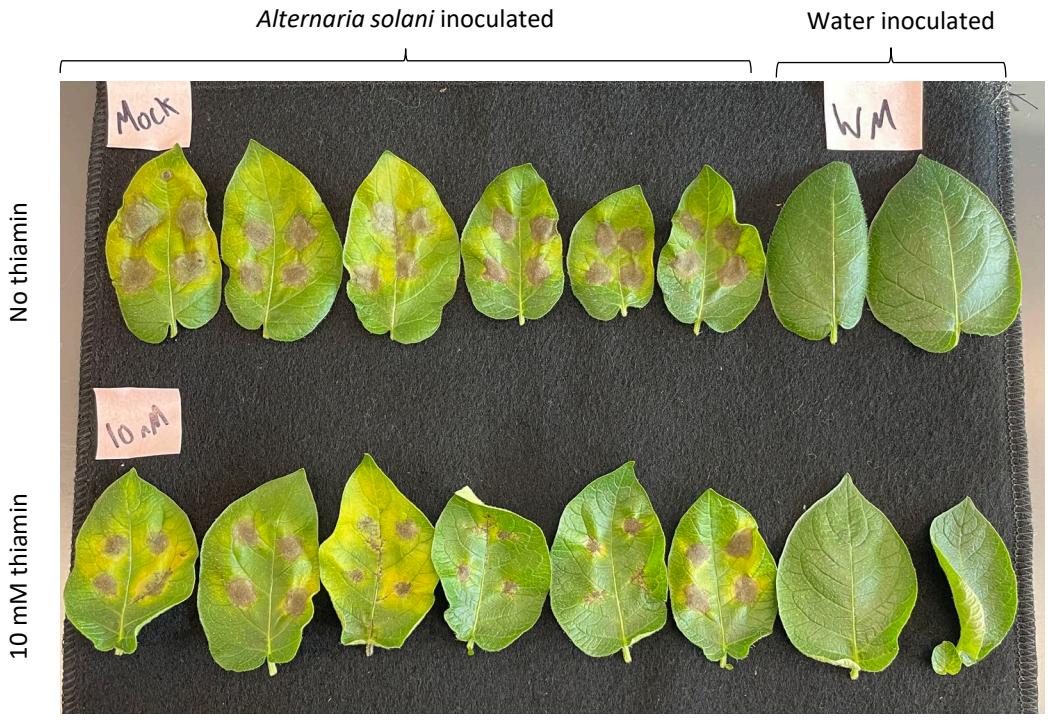

Figure S2. Representative pictures of lesions 3 days after inoculation with *A. solani* in plants treated with 10 mM thiamin or a mock solution.

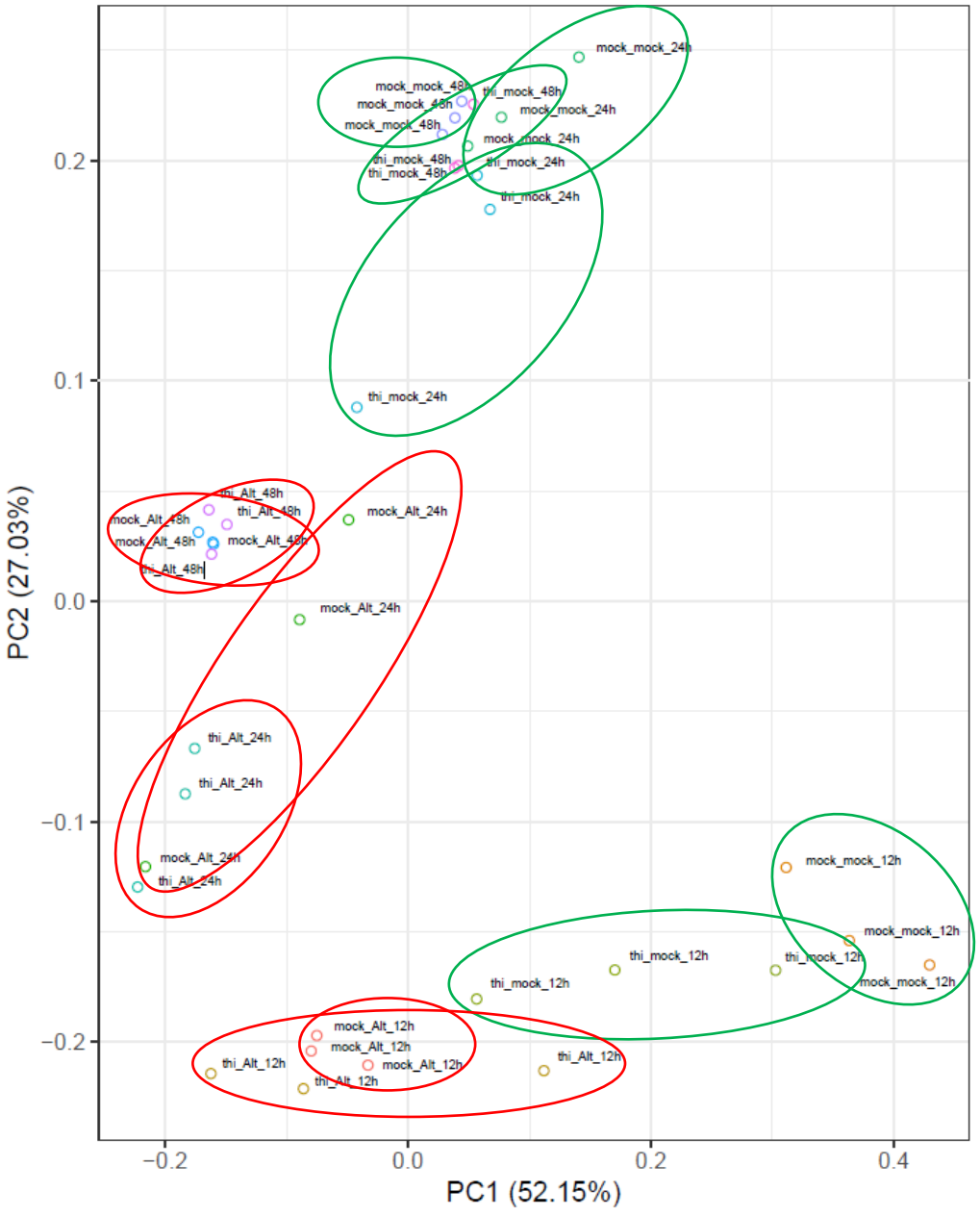

Figure S3. Principal Component Analysis (PCA) plot of RNA-seq samples. Replicates from the same treatments are circled. Red circles indicate *A. solani*-inoculated samples while green circles indicate non-inoculated samples.

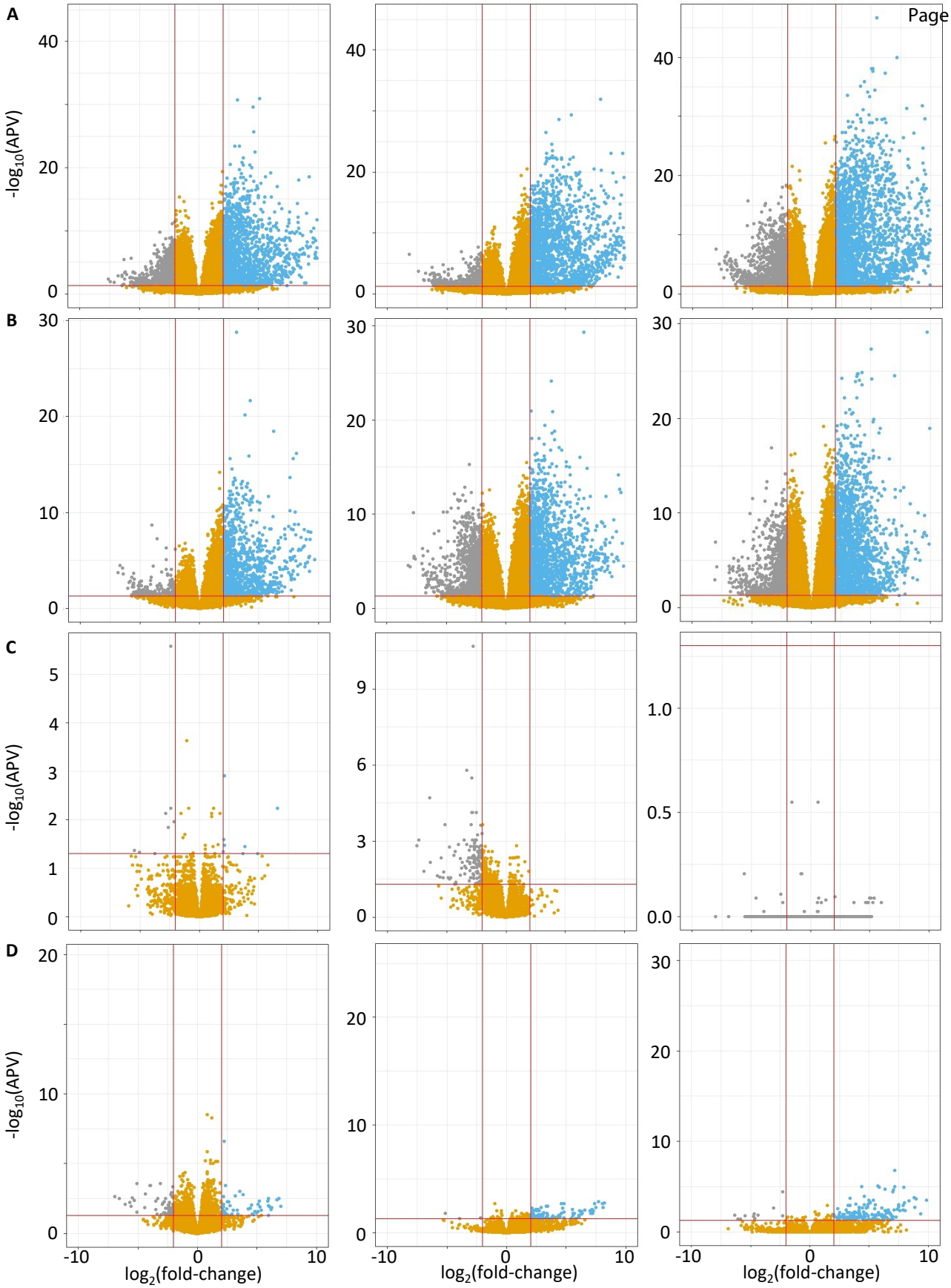

Figure S4. Volcano plots for differentially expressed genes (DEGs) at 12 (left), 24 (center), and 48 (right) hours post-inoculation. (A), "response to Alt (local)." (B), "response to Alt (thi)." (C), "response to thi (Alt)." (D), "response to thi." "APV", adjusted p-value.

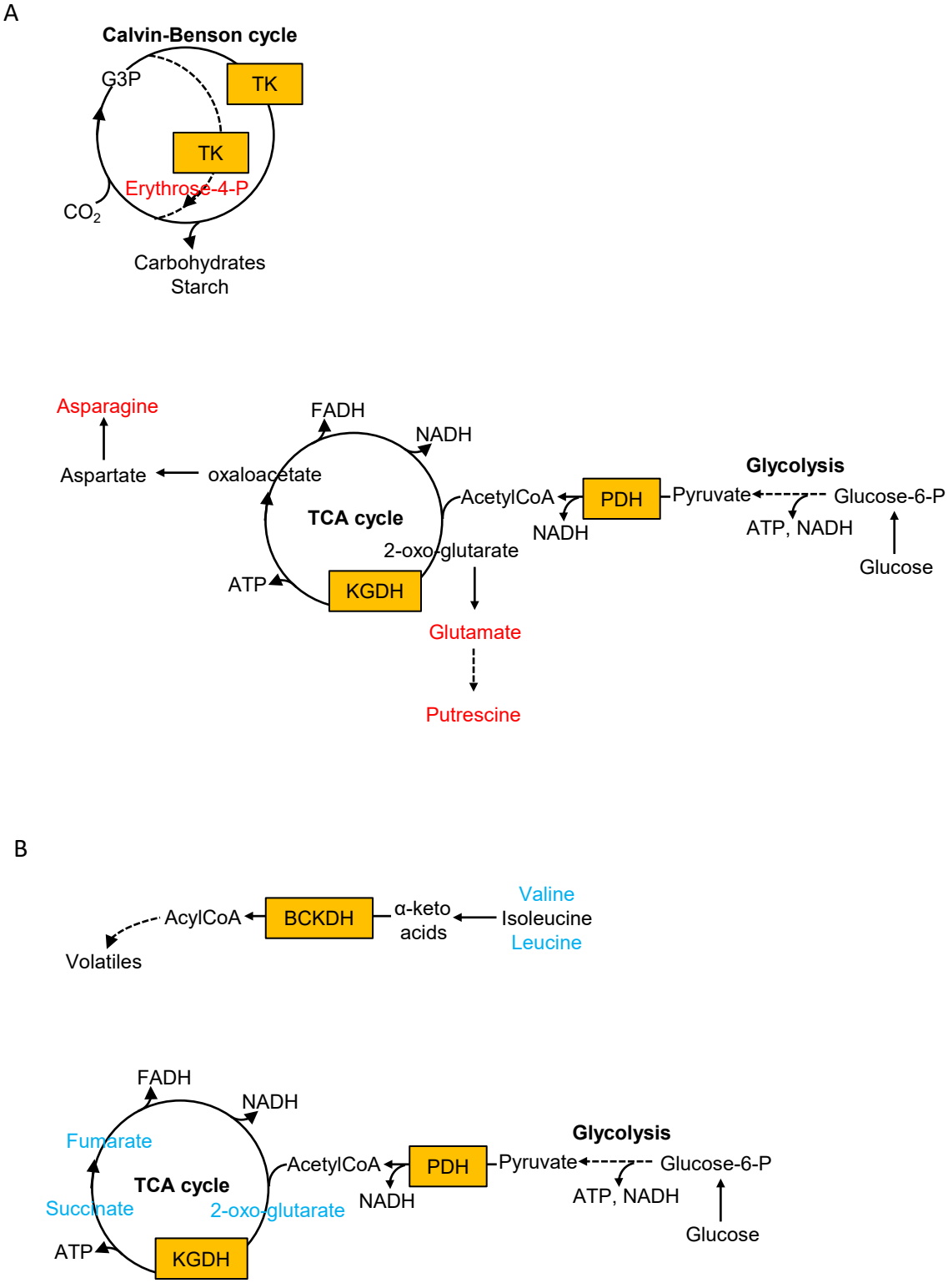

Figure S5. Simplified schema of the Calvin cycle, glycolysis, the TCA cycle, and α-ketoacids catabolism with thiamin-dependent enzymatic steps. In orange squares are thiamin-dependent enzymes. TK, transketolase; PDH, pyruvate dehydrogenase; KGDH, 2-oxoglutarate (α-KG) dehydrogenase; BCKDH, branched-chain amino acids ketodehydrogenase. A. Thiamin-dependent pathways where metabolites accumulated at 1 hpt with thiamin (in red text). B. Thiamin-dependent pathways where metabolites decreased at 6 hpt with thiamin (in blue text).