

Temporal multi-omics dataset of a cucurbit–powdery mildew interaction

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Abstract

Plant-pathogenic fungi are major contributors to global crop losses, yet many of the most damaging groups, especially obligate biotrophs, remain difficult to study. Their strict dependence on living host tissue limits experimental manipulation and has constrained the generation of comprehensive datasets that capture how these pathogens suppress host immunity and reprogram metabolism over time. To expand the resources available for data-driven research, we present a temporally resolved multi-omics dataset characterizing the interaction between *Cucumis melo* and *Podosphaera xanthii*, the causal agent of cucurbit powdery mildew and a model obligate biotroph. Inoculated and mock-treated melon leaves were sampled across 12 time points from 12 to 144 hours post inoculation, covering key developmental transitions of the pathogen. The dataset integrates dual RNA-seq and untargeted LC–MS/MS metabolomics generated from the same biological material, accompanied by detailed documentation of experimental design, bioinformatic workflows, quality-control metrics and processed outputs to ensure broad reusability. This resource enables exploration of host–pathogen transcriptional dynamics, stage-specific infection responses, metabolic remodeling and integrative multi-omics analyses of obligate biotrophy.

Background & Summary

Powdery mildew fungi (*Erysiphales*) are among the most widespread and economically damaging plant pathogens. These obligate biotrophic fungi infect more than 10,000 host species, including cereals, grapevine, legumes, solanaceous crops, fruit trees and numerous ornamentals (Braun et al., 2002; Glawe, 2008). Cucurbits are particularly affected, with *Podosphaera xanthii* recognized as the primary causal agent of cucurbit powdery mildew and a major constraint on global production (del Pino et al., 2002; Fernández-Ortuño et al., 2006). Although chemical fungicides remain the cornerstone of disease management, their long-term efficacy is increasingly undermined by the rapid emergence of resistant populations. Accordingly, the Fungicide Resistance Action Committee (FRAC) classifies *P. xanthii* as a high-risk pathogen for fungicide resistance development (Vielba-Fernández et al., 2020).

As obligate biotrophs, powdery mildew fungi must complete their entire life cycle on living host tissue, establishing intimate associations with plant cells through haustoria. These specialized structures mediate nutrient uptake and deliver effectors that reprogram host metabolism and suppress immune responses (Kemen et al., 2015; Polonio et al., 2019a; Polonio et al., 2020). This lifestyle imposes substantial experimental constraints: unlike many fungal pathogens, powdery mildews cannot be cultured on artificial media, limiting genetic manipulation and functional validation (Pérez-García et al., 2009). Recent methodological advances, including RNA interference-based gene silencing (Ruiz-Jiménez et al., 2021; Padilla-Roji et al., 2026) and surrogate model systems using closely related species (Loos et al., 2025), have expanded the available toolkit. Nevertheless, comprehensive *in planta* datasets remain essential for dissecting the molecular basis of biotrophy and the dynamic host–pathogen interactions that sustain infection.

High-throughput omics technologies have provided valuable insights into plant–powdery mildew interactions. However, most available time-course datasets include only a few sampling points and rely predominantly on transcriptomics (Table 1), limiting systems-level analyses. To address the gap, we generated a temporally resolved multi-omics resource of the *Cucumis melo*–*Podosphaera xanthii* interaction (Fig. 1). To our knowledge, this dataset constitutes the first time-resolved resource integrating dual transcriptomics and untargeted metabolomics for a plant host interacting with an obligate biotrophic fungal pathogen. By capturing coordinated transcriptional and metabolomic changes across infection, it provides a foundation for investigating the molecular basis of biotrophy, reconstructing host–pathogen metabolic exchanges, identifying candidate genes and pathways associated with disease progression, and enabling comparative analyses across diverse plant–pathogen systems. All raw data,

metadata and processed outputs are publicly available to facilitate reuse and integration in future studies of plant immunity, fungal pathogenicity and host–pathogen interactions.

Table 1 Comparative overview of available time-course omics datasets for plant–powdery mildew interactions.							
Reference	Host	Powdery mildew	Omics	Range (hpi)	Sampling points	Focus	Accession
This study	<i>C. melo</i>	<i>P. xanthii</i>	Transcriptomics and metabolomics	12–144	12	Multi–omics resource	PRJNA1454108 MTBLS15447
Bonarota et al., 2025	<i>V. vinifera</i>	<i>E. necator</i>	Transcriptomics	24–240	3	Cell–type atlas	PRJNA1295527
Qian et al., 2023	<i>H. vulgare</i>	<i>B. hordei</i>	Transcriptomics	0–144	7	Fungal regulatory networks	PRJNA835302
Polonio et al., 2019b	<i>C. melo</i>	<i>P. xanthii</i>	Transcriptomics	24–72	3	Host responses	PRJNA434538
Hu et al., 2018	<i>T. aestivum</i>	<i>B. graminis</i> f. sp. <i>tritici</i>	Transcriptomics	0–72	6	Pathogenesis	PRJNA401295

Abbreviation: hpi, hours post inoculation

Table 2. Sequencing depth and mapping statistics for the dual RNA-seq dataset. Summary of sequencing output, read filtering and read assignment statistics for inoculated and mock-treated samples. Values represent the median number of paired-end read pairs per library, with ranges in parentheses. Complete per-library statistics are provided in Supplementary Table S1.

Metric	Inoculated samples	Mock samples
Raw reads	80.7 M (3.6–198.3 M)	70.8 M (3.8–218.4 M)
Filtered reads	71.8 M (2.4–167.3 M)	58.1 M (2.8–179.4 M)
Reads mapped to <i>C. melo</i>	66.6 M (12.2–164.6 M)	57.4 M (2.8–177.5 M)
Reads mapped to <i>P. xanthii</i>	2.07 M (0.02–12.4 M)	NA

Abbreviation: NA, not applicable.

Methods

Fungal isolate and plant material

The *Podosphaera xanthii* isolate 2086 was routinely propagated on detached zucchini cotyledons (*Cucurbita pepo* L. cv. ‘Negro Belleza’; Semillas Fitó, Barcelona, Spain) and maintained *in vitro* on Bertrand medium in 8-cm Petri dishes (Álvarez & Torés, 1997). Cultures were incubated at 24°C under a 16-h light/8-h dark photoperiod. For infection assays, melon plants (*Cucumis melo* L. cv. ‘Rochet’; Semillas Fitó), a cultivar susceptible to cucurbit powdery mildew,

were grown for four weeks in a controlled-environment growth chamber under identical photoperiod and temperature conditions prior to inoculation.

Experimental design

Cotyledons and the first two true leaves were excised from melon plants, which were then staked to maintain upright growth. They were transferred to two separate GROW-1300 HR growth chambers (ENTERLAB, Terrassa, Spain), one per condition, equipped with LED T8 tube lighting (Magno, 18 W, 6000 K, 1710 lm). Plants were acclimated for two days under the conditions described above and 90% relative humidity. Inoculum was prepared by immersing infected zucchini cotyledons in sterile distilled water containing 0.01% (v/v) Tween-20. The third and fourth true leaves were spray-inoculated with a conidial suspension adjusted to 1×10^6 conidia mL⁻¹. Mock-treated plants received 0.01% (v/v) Tween-20 only. Leaf tissue was collected every 12 h from 12 to 144 h post-inoculation (hpi), generating twelve time points spanning the complete infection cycle. This experimental design captured successive developmental transitions of the pathogen and the associated host responses. Sampling alternated between dark and light conditions, beginning with the dark period at 12 hpi. A total of 72 plants were used (36 inoculated, 36 mock). For each treatment and time point, three independent biological replicates were harvested, each consisting of one leaf from a different plant. Samples were immediately flash-frozen in liquid nitrogen, ground to a fine powder using a pre-chilled mortar and pestle and stored at -80°C until RNA and metabolite extraction. The same biological samples were subsequently profiled by dual RNA-seq and untargeted LC-MS/MS metabolomics (Fig. 1a). All plants were grown, inoculated and sampled within a single experimental batch to minimize potential batch effects.

Visualization of fungal development

Progression of the *P. xanthii* life cycle was monitored using lactophenol cotton blue (LPCB) staining. Leaf discs (8 mm diameter) were collected at each sampling time point and incubated overnight at room temperature in the dark in LPCB:ethanol (1:100, v/v). Samples were cleared by two washes with acetic acid:ethanol (1:3, v/v), destained overnight in the same solution and stored in 70% ethanol until microscopic observation. Fungal structures were visualized using an Eclipse E800 light microscope (Nikon, Tokyo, Japan), to characterize the major developmental stages of the pathogen, from early host penetration events to extensive mycelial growth and sporulation (Fig. 1b).

Transcriptomics

Total RNA was extracted using TRI Reagent (Sigma-Aldrich, St. Louis, MO, USA) following the manufacturer's protocol. RNA concentration and purity were assessed using a Qubit 3.0 Fluorometer and a NanoDrop One spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), respectively. RNA integrity was evaluated using an Agilent 2100 Bioanalyzer with the Plant RNA Nano kit (Agilent Technologies, Santa Clara, CA, USA). Stranded mRNA libraries were prepared using the Illumina Stranded mRNA Prep kit (Illumina, San Diego, CA, USA) and their quality was checked using an Agilent 2100 Bioanalyzer with the High Sensitivity DNA Kit. Libraries were sequenced as 2 x 100 bp paired-end reads on an Illumina NovaSeq 6000 platform.

The transcriptomic pipeline is summarized in Fig. 2a. Raw reads were quality-checked with FastQC (v0.11.9) (Babraham Bioinformatics, UK) and MultiQC (v1.27) (Ewels et al., 2016). Adapter removal and quality trimming were performed using Trimmomatic (v0.39) (Bolger et al., 2014) with parameters: ILLUMINACLIP:2:30:10, LEADING:28, TRAILING:28, SLIDINGWINDOW:4:28, MINLEN:35. Filtered reads were re-evaluated using FastQC and MultiQC. For mock samples, reads were aligned to the *Cucumis melo* reference genome assembly v4.0 (Castanera et al., 2020) using HISAT2 (v2.2.1) (Kim et al., 2015). For inoculated samples, reads were first aligned to the melon genome;

unmapped read pairs were then aligned to the *P. xanthii* isolate 2086 genome (Polonio et al., 2021). Alignment files were processed using SAMtools (v1.23.1) (Li et al., 2009). Read counts were generated with featureCounts (v2.0.6) (Liao et al., 2014) in paired-end mode using exon features of the corresponding GFF3 annotations. Differential expression analyses were performed using DESeq2 (v1.46.0) (Love et al., 2014). Differentially expressed genes (DEGs) were defined as those with adjusted p-value < 0.05 (Benjamini–Hochberg correction) and $|\log_2\text{FC}| > 1$. Principal component analysis (PCA) was conducted on DESeq2 rlog-transformed counts for both *C. melo* and *P. xanthii* to assess global transcriptional variation. Functional enrichment of *C. melo* genes was performed using DAVID (v6.8) (Sherman et al., 2022) based on KEGG annotations. For *P. xanthii*, enrichment analyses were conducted using clusterProfiler (v4.14.6) (Wu et al., 2021) with Gene Ontology (GO) terms assigned via eggNOG-mapper (v2) (Cantalapiedra et al., 2021), which provided broader functional coverage for the fungal gene set.

Metabolomics

Samples collected during the infection assay were lyophilized prior to metabolite extraction. Metabolites were extracted from 15–30 mg of lyophilized leaf powder in 1 mL of 30% (v/v) methanol containing 0.01% (v/v) formic acid. Samples were homogenized with glass beads in a TissueLyser (Qiagen, Hilden, Germany) at 30 Hz for 2 min and centrifuged at $14,000 \times g$ for 20 min at 4°C. Supernatants were filtered through 0.22- μm regenerated cellulose syringe filters (Phenomenex, Torrance, CA, USA). For untargeted metabolomics, three biological replicates and two technical replicates per biological sample were analyzed. Extracts were diluted 1:4 and analyzed using an ACQUITY UPLC I-Class system (Waters, Milford, MA, USA) coupled to a SYNAPT G2-S HDMS Q-TOF mass spectrometer (Waters). Chromatographic separation was performed on a Kinetex C18 column (2.6 μm , 50×2.1 mm; Phenomenex) using a water–methanol gradient with 0.01% (v/v) formic acid at 0.3 mL min⁻¹. Injection volume was 5 μL . The analytical sequence included solvent blanks and calibration standards (5 and 10 $\mu\text{g L}^{-1}$). Mass spectra were acquired in ESI+ and ESI- modes over m/z 40–1,200, with blank injections between modes to monitor carryover.

The metabolomics workflow is shown in Fig. 2b. Raw LC–MS/MS data were acquired using MassLynx (v4.2) (Waters) and converted to NetCDF using DataBridge. Data processing was performed with the xcms package (Bioconductor), including centWave peak detection, retention time correction, peak grouping and missing-value imputation. Intensities were normalized to sample dry weight and technical replicates were averaged. Processed feature tables were imported into MarVis-Suite 2.0 (Kaeffer et al., 2015) via MarVis-Filter, where intensities were log-transformed and median scaled. Differentially accumulated features were identified using one-way analysis of variance (ANOVA) with Benjamini–Hochberg FDR correction. Isotope and adduct correction used a maximum of two ¹³C isotopes, cosine similarity ≥ 0.75 , retention time tolerance 10 s and m/z tolerance 0.01 Da. Features with adjusted p-value < 0.05 were retained. Principal component analysis (PCA) was performed on significant features to assess dataset structure. To visualize temporal dynamics, the 20 most variable features were selected, averaged across biological replicates and standardized by z-score prior to heatmap visualization. Filtered features were imported into MarVis-Pathway for tentative metabolite annotation and pathway enrichment. Putative annotations were assigned by matching accurate mass and retention time information against three in-house metabolite libraries (Gamir et al., 2014), following recommendations for reporting metabolite identification confidence (Schymanski et al., 2014; Alseekh et al., 2021). Enriched KEGG pathways were summarized based on pathway scores, metabolite hits and pathway coverage, excluding broad global categories.

Data Records

The RNA-seq dataset (BioProject PRJNA1454108) comprises paired-end sequencing reads generated from mock-treated and *Podosphaera xanthii*-inoculated melon samples collected across 12 time points spanning 12–144 hpi.

Three biological replicates were obtained for each condition and time point, resulting in 72 paired-end libraries (144 FASTQ files). Sample-level metadata, sequencing statistics and library preparation details are provided to support downstream reuse and reproducibility.

The metabolomics dataset (MetaboLights MTBLS15447) includes raw LC–MS/MS data acquired in both positive and negative electrospray ionization modes (ESI + and ESI–), together with converted NetCDF files, sample metadata and full acquisition parameters. The experimental design comprised two conditions (mock-treated and inoculated), 12 time points, three biological replicates per condition and time point, two technical replicates per biological sample and two ionization modes, yielding 288 raw LC–MS/MS files. Corresponding MS1 and MS2 spectra are provided as 576 NetCDF files, enabling the use of fragmentation data to support metabolite annotation and increase confidence in compound identification.

Additional processed multi-omics datasets are available through Zenodo at <https://zenodo.org/records/19555639>. These resources include gene count matrices, TPM expression matrices, differential expression, functional enrichment outputs, processed metabolomic feature tables, statistically significant metabolomic features, putative metabolite annotations, pathway enrichment results and complete sample metadata. Together, these files facilitate data reuse, integrative analyses and cross-study comparisons within plant–pathogen interaction research.

Technical Validation

Transcriptomics

RNA quality and integrity were evaluated prior to library preparation to ensure suitability for RNA-seq. Purity was assessed spectrophotometrically using A260/A280 and A260/A230 ratios and integrity was examined by electrophoresis on 2% agarose gels and by calculating the RNA Integrity Number (RIN). All samples exhibited RIN values ≥ 7 , meeting the threshold for high-quality library construction; one sample below this threshold was re-extracted and re-evaluated. Final RNA concentrations were quantified using a Qubit fluorometer to ensure accurate input amounts.

Quality-control analyses confirmed overall high sequencing performance across the RNA-seq libraries (**Fig. S1a**). The dataset comprised 5,811,479,164 raw paired-end reads, of which 4,736,897,834 (81.5%) were retained after filtering. Median Phred scores exceeded Q35 across all read positions, indicating high base-calling accuracy (**Fig. S1b**). Filtered reads were aligned to the *Cucumis melo* and *Podosphaera xanthii* genomes as described in the Methods. In total, 4,558,311,107 read pairs mapped to *C. melo* and 98,195,334 to *P. xanthii*. Alignment statistics are summarized in **Table 2**, with per-sample details provided in **Table S1**. One inoculated sample at 36 hpi (36h_I_R3) showed lower sequencing depth (3.6 million raw read pairs) than the other inoculated libraries. However, quality metrics were comparable, and the sample was retained (**Table S1**). Given the low number of fungal reads (19,092 paired-end reads), fungal expression results for this sample should be interpreted cautiously.

Principal component analysis (PCA) of rlog-transformed counts confirmed the expected biological structure of the dataset (**Fig. 3a,b**). For *C. melo*, PC1 (50%) captured temporal variation and light phase, while PC2 (27%) separated mock and inoculated samples (**Fig. 3a**). For *P. xanthii*, PC1 (43%) primarily reflected infection stage, with the first two principal components explaining 60% of total variance (**Fig. 3b**). Samples at 108 hpi formed a distinct cluster, indicating a unique transcriptional state during late infection (**Fig. 3b**). Biological replicates clustered tightly across both species, supporting high reproducibility.

Temporal patterns in read assignment further validated dataset consistency (**Fig. 3c**). The proportion of reads mapping to *C. melo* decreased from 97.7% at 12 hpi to 80.2% at 144 hpi, while *P. xanthii* reads increased from 0.65% to 18.0%, mirroring fungal colonization dynamics observed microscopically (**Fig. 1b**). Gene detection was consistently high for *C. melo*, with medians of 15,630 (mock) and 16,074 (inoculated) genes per sample (**Fig. 3d**). For *P. xanthii*, a median of 8,666 genes was detected across inoculated samples, with greater variability reflecting differences in fungal biomass (**Fig. 3d**).

Functional enrichment provided an additional layer of biological validation (**Fig. 4**). For *C. melo*, enriched KEGG pathways were identified by comparing inoculated and mock samples at each time point (**Fig. 4a**). This approach revealed temporally structured enrichment patterns, with phenylpropanoid biosynthesis, phenylalanine metabolism and plant hormone signaling enriched throughout. For *P. xanthii*, enriched GO Biological Process terms across consecutive infection stages captured key processes associated with fungal growth and host colonization, including filamentous growth, symbiotic interaction, transport, cellular homeostasis and developmental transitions (**Fig. 4b**). Together, these results confirm the biological coherence of the transcriptomic dataset and its suitability for investigating transcriptional dynamics during obligate biotrophy.

Metabolomics

Technical accuracy of the metabolomics dataset was ensured through the inclusion of calibration standards (5 and 10 $\mu\text{g L}^{-1}$), solvent blanks and technical replicates throughout the LC–MS/MS workflow. Calibration standards verified instrument performance, blanks monitored carryover and technical replicates assessed analytical reproducibility.

Untargeted LC–MS/MS analysis detected 3,901 features (ESI⁻) and 8,215 features (ESI⁺). After isotope correction, adduct grouping and statistical filtering, 187 (ESI⁻) and 165 (ESI⁺) features were retained, yielding 352 significant metabolic features. PCA of these features revealed clear separation between inoculated and mock samples along PC1 (38% of variance), with mock samples forming a compact cluster and inoculated samples showing a broader dispersion along PC1 and PC2 (9%), consistent with extensive metabolic remodelling during infection (**Fig. 5a**).

The 20 features with the highest temporal variance displayed distinct abundance trajectories between treatments (**Fig. 5b**). Mock samples exhibited stable profiles, whereas inoculated samples showed progressive increases in feature abundance at later time points, consistent with infection-associated metabolic reprogramming.

Putative annotations were assigned to 89 features, which mapped to 67 metabolic pathways. Enriched pathways included phenylpropanoid biosynthesis, phenylalanine and tyrosine metabolism, ubiquinone and other terpenoid-quinone biosynthesis, and amino acid metabolism, processes commonly associated with plant responses to biotic stress (**Fig. 5c**). These results demonstrate that the metabolic dataset provides sufficient resolution to capture infection-associated metabolic dynamics across the disease cycle.

Declarations

Data Availability

The datasets generated in this study are deposited in publicly accessible repositories. Raw RNA-seq data are available in the NCBI Sequence Read Archive (SRA) under BioProject accession PRJNA1454108. Raw LC–MS/MS metabolomics data are available through MetaboLights under accession MTBLS15447. Processed datasets, metadata tables, annotation files and supplementary materials are archived in Zenodo and are available at <https://zenodo.org/records/19555639>.

Code Availability

Custom scripts used for transcriptomic and metabolomic analyses are publicly available on GitHub at <https://github.com/alexjisa/PxCm-multiomics>. Data processing was performed in R (v4.4.2), and all software packages, versions and key parameters are detailed in the Methods section.

Author Contributions

A.J.-S., D.F.-O., Á.P. and A.P.-G. designed the study. A.J.-S. performed the experiments and analyzed the data. Á.P. contributed to transcriptomic data generation and analysis, and V.P. contributed to metabolomic data generation and analysis. A.J.-S., Á.P. and A.P.-G. wrote the manuscript. D.F.-O., Á.P. and A.P.-G. supervised the study and critically revised the manuscript. All authors reviewed and approved the final version.

Competing Interests

The authors declare no competing interests.

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Figures

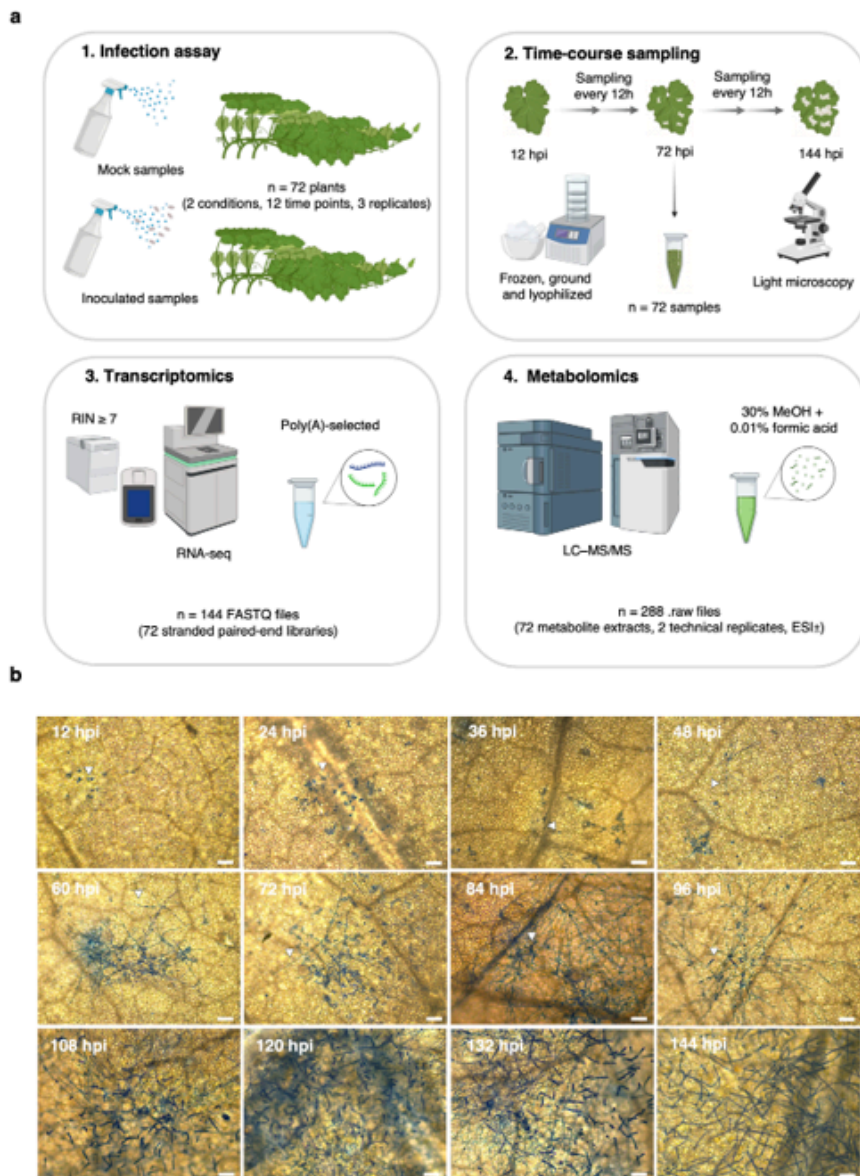


Figure 1

Experimental overview and progression of the *Cucumis melo*–*Podosphaera xanthii* interaction. (a) Schematic workflow of the multi-omics approach. Melon plants were either mock-treated or inoculated with powdery mildew and sampled every 12 h from 12 to 144 hours post inoculation (hpi). The same samples were processed for dual RNA-seq and untargeted LC–MS/MS analyses, generating a time-series dataset spanning the complete interaction cycle. (b) Representative light microscopy images showing the progression of *P. xanthii* colonization on melon leaves throughout the time course. Arrowheads indicate representative fungal structures observed at different developmental stages. Early infection included conidial germination, appressorium formation and primary hyphal growth (12–36 hpi), followed by secondary mycelial expansion and the onset of conidiogenesis (48–84 hpi), and abundant conidiophore formation with extensive mycelial proliferation at later stages (96–144 hpi). Scale bar: 100 μ m.



Figure 2

Bioinformatics workflows for omics data processing. (a) Dual RNA-seq analysis flowchart for generating gene count matrices from mock-treated melon, powdery mildew-inoculated melon and *P. xanthii* during infection. **(b)** Untargeted LC–MS/MS analysis pipeline for obtaining metabolome profiles of control and infected melon plants.

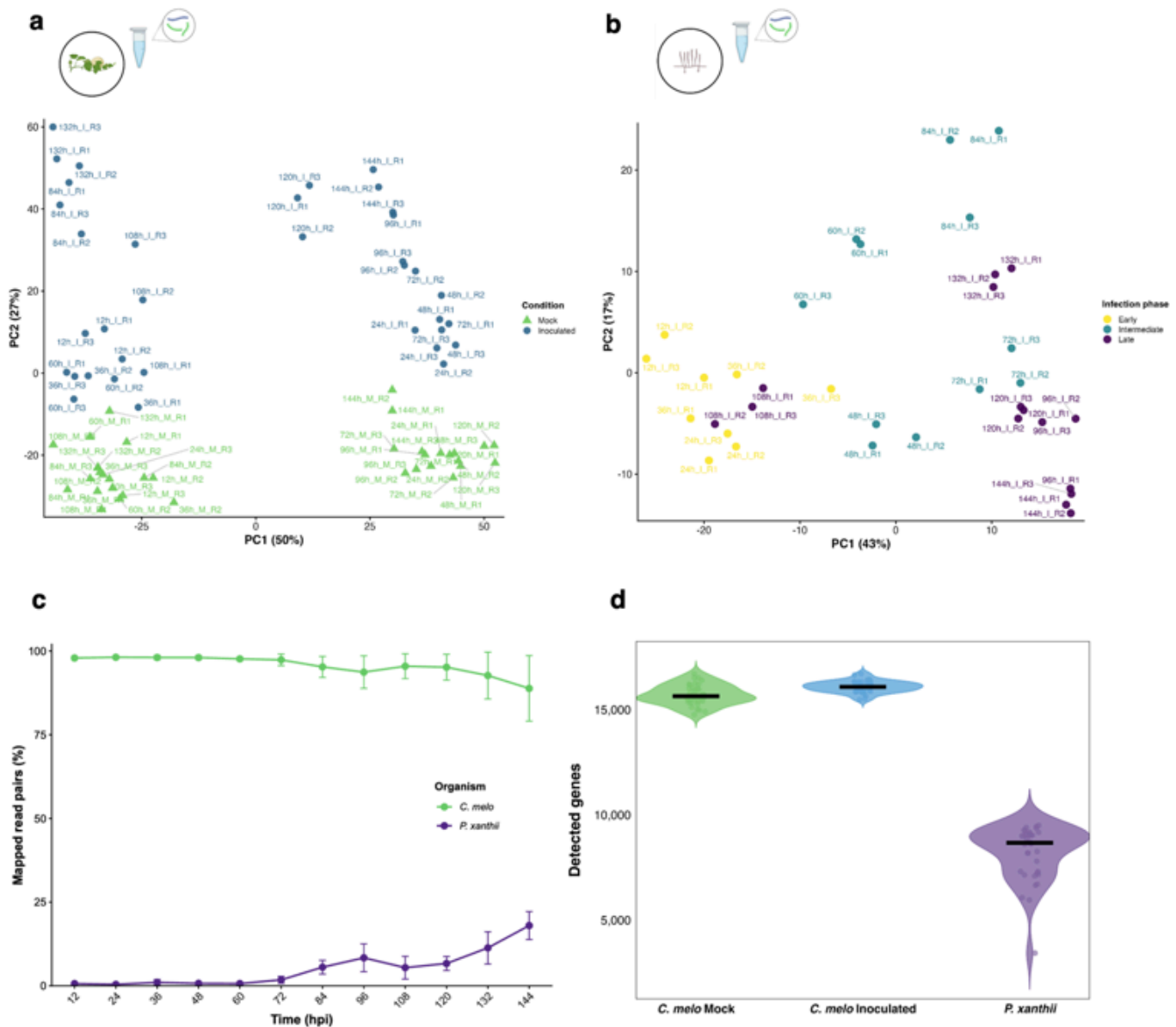


Figure 3

Technical validation of the transcriptomic dataset. (a) Principal component analysis (PCA) of gene expression profiles from *C. melo* samples, colored by condition (mock and inoculated). (b) PCA of gene expression profiles from *P. xanthii* samples, with samples colored according to the infection phase (early, intermediate and late). (c) Temporal dynamics of relative read assignment to the *C. melo* and *P. xanthii* genomes throughout the infection time course. Each time point includes three biological replicates and data are shown as mean $\pm$ standard deviation (SD). (d) Distribution of expressed genes per sample (≥ 1 TPM) in mock-treated *C. melo*, inoculated *C. melo* and *P. xanthii* samples. Violin plots show the data distribution and the horizontal black bar indicates the median.

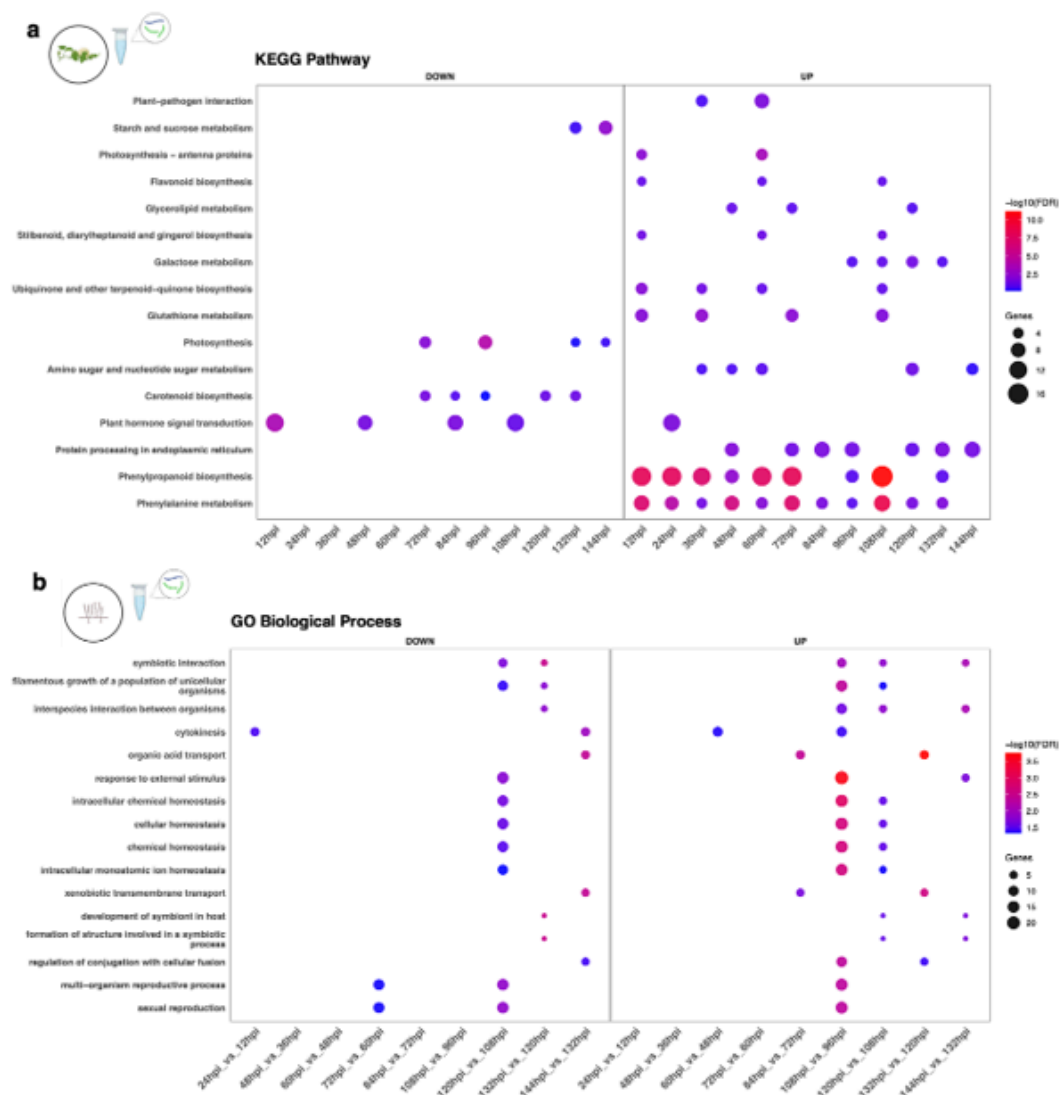


Figure 4

Functional validation of host and pathogen transcriptomes. Bubble size represents the number of differentially expressed genes (DEGs) per category; color indicates enrichment significance ($-\log_{10}$ FDR). Enriched categories are shown separately for downregulated and upregulated genes. **(a)** KEGG pathway enrichment analysis of *C. melo* DEGs between inoculated and mock-treated samples at each time point. **(b)** Gene Ontology (GO) Biological Process enrichment analysis of *P. xanthii* DEGs between consecutive infection time points.

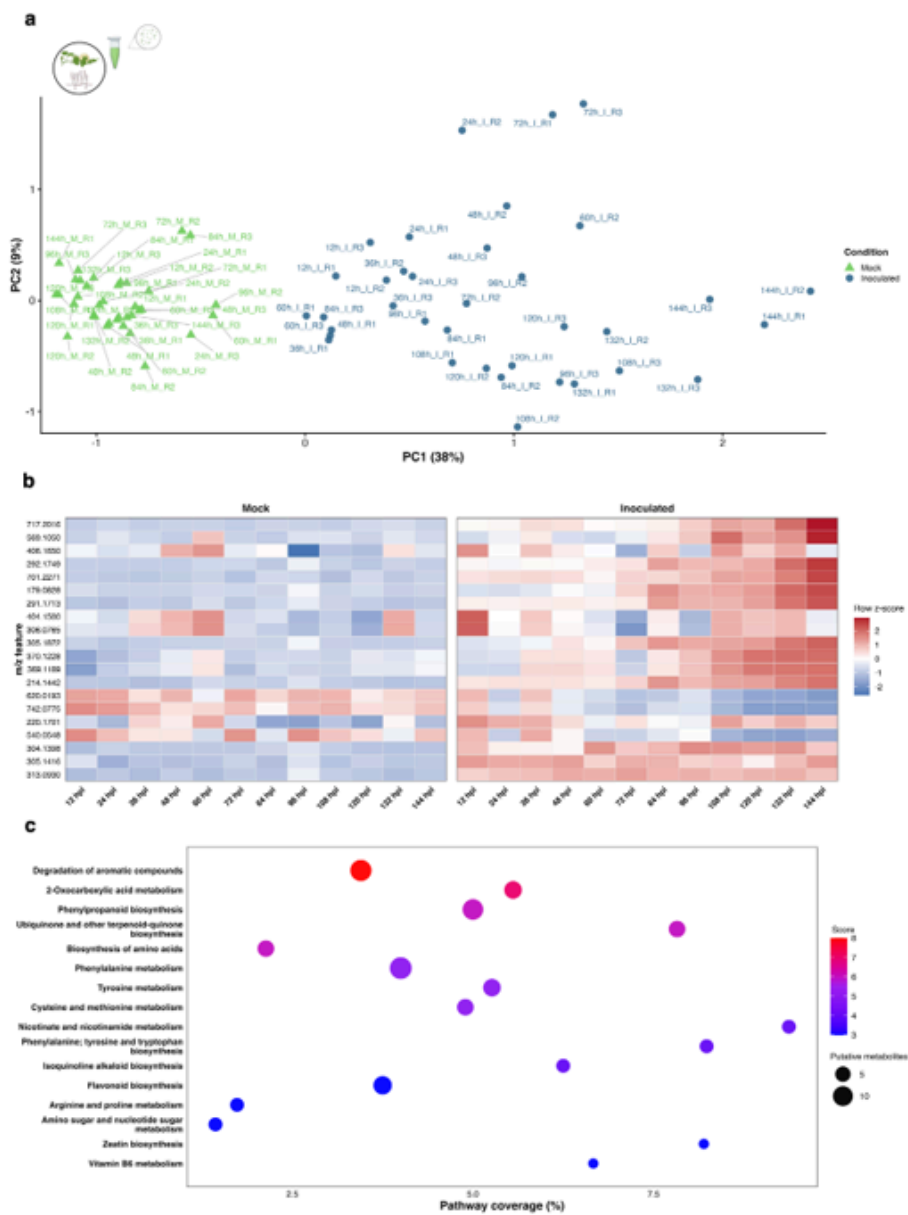


Figure 5

Technical and functional validation of the metabolomic dataset.

(a) Principal component analysis (PCA) of metabolomic features retained after statistical filtering. Samples are colored by treatment (mock and inoculated). (b) Heatmap showing the 20 metabolomic features with highest temporal variance. Feature intensities were scaled using row-wise z-scores. (c) KEGG pathway enrichment analysis of putatively annotated metabolites. Bubble size represents the number of putative metabolites associated with each pathway, color indicates the pathway score and the x-axis shows pathway coverage (% of pathway entries represented by detected metabolites).

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