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Data Article

Proteomics dataset on detached and purified *Arabidopsis thaliana* rosette leaf trichomes

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ABSTRACT

Trichomes are highly specialized uni- or multicellular outgrowths of epidermal cells of plant organs that, in the case of leaves, contribute to plant resistance against abiotic and biotic stress. The model plant *Arabidopsis thaliana* features single-celled non-glandular rosette leaf trichomes that are dispensable under laboratory conditions. Trichomes have therefore become a successful model to identify plant genes involved in cellular differentiation and cell wall development. We have recently devised an improved method for the enrichment of plant leaf trichomes that relies on the biochemical weakening of the trichome-leaf junctions and a magnetic stirrer-based mechanical stimulus for trichome release followed by density gradient purification of trichomes. Here we provide detailed information on a label-free quantitative (LFQ) shotgun proteomics dataset collected at four stages while applying this protocol to isolate trichomes from rosette leaves of *A. thaliana*, from (i) whole seedlings before enrichment, from (ii) trichome-depleted material after separa-

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tion, from (iii) detached trichomes, and from (iv) enriched trichomes after sucrose density gradient centrifugation. Proteins were extracted, digested with trypsin and the resulting peptides identified by nanoflow-chromatography coupled to tandem mass spectrometry. This dataset informs on proteins and biochemical processes present and/or enriched in *A. thaliana* rosette leaf trichomes, complementing recent large-scale proteome maps. The data further enables comparative analysis with trichome proteomic data from other plant species, may be reanalyzed using different software packages or search settings, and may serve as a reference benchmark for future method refinement.

Specifications Table

Subject	Plant Science: General
Specific subject area	Plant tissue proteomics
Type of data	Mass spectrometry data Tables Figures
How the data were acquired	Proteomes were extracted from (i) whole seedlings before enrichment, from (ii) trichome-depleted material after separation using a magnetic stirrer in the presence of ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA), from (iii) crude rosette leaf trichomes and from (iv) enriched trichomes after subsequent purification using sucrose density gradients. Proteins were digested, the resulting peptides desalted using in-house packed C18-STAGE tips and analyzed by tandem mass spectrometry using an Ultimate 3000 RSLCnano liquid chromatography system (Thermo) coupled to an Impact II quadrupole time-of-flight (QqTOF) mass spectrometer (Bruker) using a CaptiveSpray nano-ESI source (Bruker) with a nanoBooster (Bruker) engaged in the nitrogen stream. Bruker Hystar software 5.1 was used to acquire the mass spectrometry raw data. Peptides and proteins were identified using MaxQuant v. 1.6.10.43 in combination with the Uniprot *A. thaliana* proteome database (release 2021_02).
Data format	Raw Analyzed Filtered
Description of data collection	Proteomes were collected from four replicates at four steps of a plant trichome isolation procedure ((ii) whole seedlings before enrichment, (ii) trichome-depleted material after mechanical separation, (iii) crude trichomes and (iv) enriched trichomes after purification using sucrose density gradients). Protein concentration was estimated using a bicinchoninic acid (BCA) assay. Digestion and mass spectrometry analysis was based on equal protein amounts as input material.
Data source location	Sample generation: Unit of Plant Molecular Cell Biology, Institute for Biology I, RWTH Aachen University, Worringerweg 1, 52,056 Aachen, Germany Mass spectrometry: Central Institute for Engineering, Electronics and Analytics, ZEA-3, Forschungszentrum Jülich, Wilhelm-Johnen-Str, 52,425 Jülich, Germany
Data accessibility	The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD028808 and can be accessed at https://www.ebi.ac.uk/pride/archive/projects/PXD028808 An exemplary data analysis comparing protein abundance in trichome-enriched fractions to trichome-depleted source material is provided as a supplementary table in the Open Science Framework repository, project name "*Arabidopsis thaliana* trichome proteome", https://osf.io/pmc24/, DOI 10.17605/OSF.IO/PMC24

(*continued on next page*)

Related research article	J W Huebbers, K Büttgen, F Leissing, M Mantz, M Pauly, P F Huesgen, R Panstruga, An advanced method for the release, enrichment and purification of high-quality *Arabidopsis thaliana* rosette leaf trichomes enables profound insights into the trichome proteome. Plant Methods. 2022 Jan 28;18(1):12.doi:10.1186/s13007-021-00,836-0 [1]

Value of the Data

- This dataset provides an updated compendium of proteomes of *A. thaliana* rosette leaf trichomes and thus will be of interest for anyone studying trichome biology
- The dataset comprises two trichome-enriched fractions of differing purity as well as the corresponding source leaf proteome and trichome-depleted leaf proteome, allowing benchmarking of future trichome isolation procedures
- The mass spectrometry raw data may be re-investigated with alternative and/or improved data analysis procedures to identify additional proteins and/or the presence of post-translationally modified peptides
- The data may be useful for meta-analysis such as comparisons across plant organs or species

1. Objective

Trichomes are specialized unicellular or multicellular structures on aerial plant organs [2]. Depending on the plant species, trichomes vary greatly in shape and morphology and comprise glandular as well as non-glandular forms. Trichomes contribute to plant protection against a variety of abiotic and biotic stresses, such as water loss, freezing tolerance, UV radiation and herbivore feeding, which often includes the synthesis, storage or secretion of specialized metabolites [3]. Their accessible, easily observable location and at least under laboratory conditions non-essential function has established trichomes as a model system to study cell development, cellular differentiation and cell wall development [2], which is increasingly also used as a target for metabolic engineering [4]. To allow for in-depth characterization of plant trichomes using systems biology “omics” approaches, we have recently established an optimized procedure for the isolation of highly purified trichomes that is applicable to a variety of plant species [1,5]. Here we provide details on a proteome dataset from the model plant *A. thaliana* that was generated as proof-of-concept during the trichome enrichment method development, allowing for the comparison between enriched trichomes, the source material and two intermediate fractions, the trichome-depleted leaf proteome and separated trichomes.

2. Data Description

We describe a quantitative *A. thaliana* proteome dataset acquired at four stages of our recently described improved plant trichome enrichment protocol [1,5]. The method includes two critical steps, (i) the physical separation of the trichomes from the leaf material using the combined action of a chelating agent and gentle stirring, followed by separation of crude trichomes from the remaining material using two consecutive filtration steps with screen door mesh and a cell strainer, and (ii) a sucrose density gradient centrifugation to further separate intact trichomes from delicate debris (Fig. 1). For proteome analysis, we collected the following sample material throughout the procedure: (i) Above-ground tissue of *A. thaliana* as source material directly after harvest with a razor blade (designated LEAF(+)), (ii) trichome-depleted leaf tissue (LEAF(-)), (iii) detached trichomes released by gentle stirring in EGTA-PBS buffer (RAW), and (iv) trichomes after further purification by sucrose density gradient centrifugation (PURE). The enrichment procedure was performed two times each from two independent batches of seedlings (replicates 1 and 2, and replicates 3 and 4, respectively). Proteomes were extracted

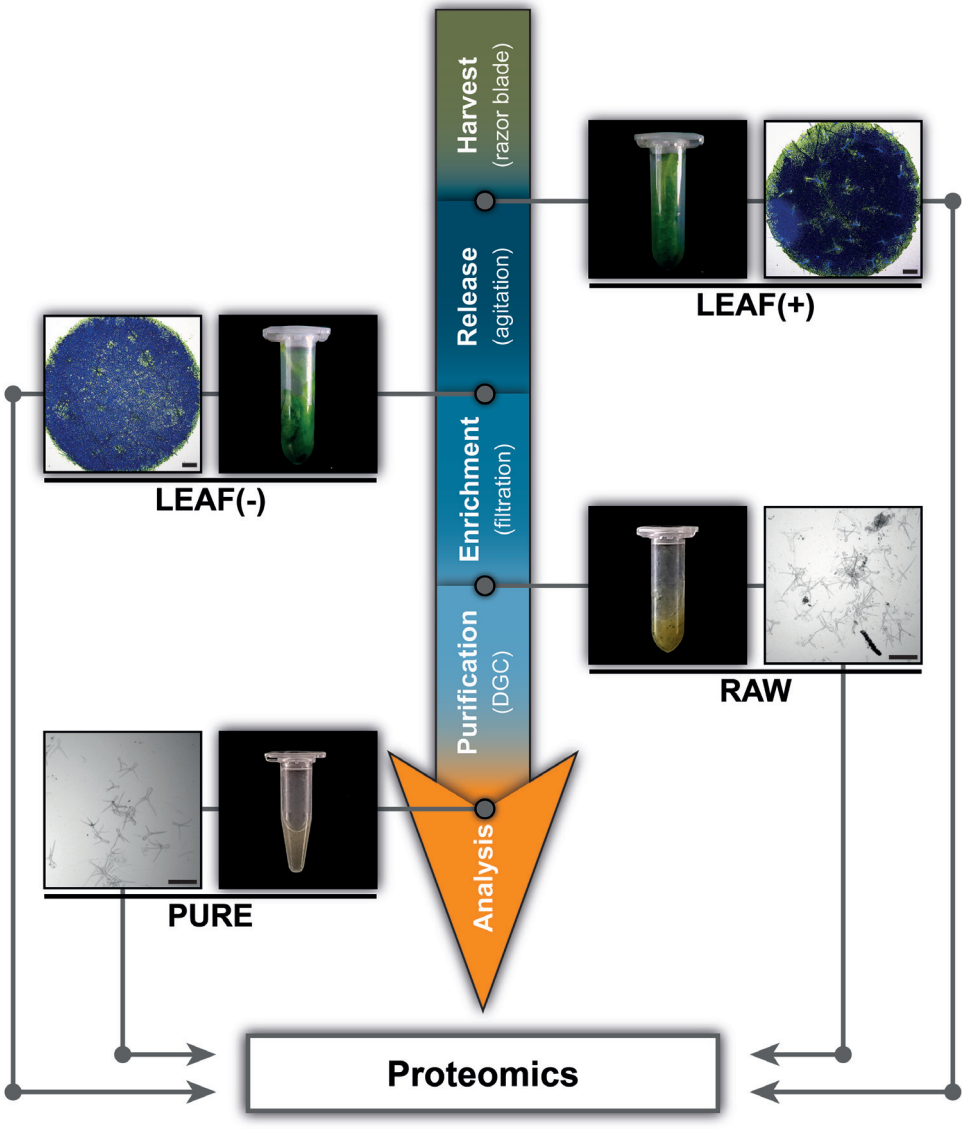

Fig. 1. Workflow scheme for the sampling of *A. thaliana* trichomes and rosette leaf reference samples for proteomic profiling. The arrow portrays the main steps during trichome isolation. The basic principle of each step is indicated in parentheses, whereas the exact methodology is described in the Materials and Methods section. Images connected to the intersections of the different steps show representative reaction tubes and micrographs of the various sample types that were subjected to proteomic analysis. Note that micrographs of leaf discs (adaxial view) are merged pictures of brightfield and ultraviolet illumination with trichomes exhibiting auto-fluorescence upon the latter. The micrographs of trichomes and leaf discs as well as the photographs of trichomes in reaction tubes were published before [1]. Scale bars represent 500 µm. DGC: density gradient centrifugation, LEAF(+): *A. thaliana* leaves before trichome release, LEAF(-): *A. thaliana* leaves after trichome release, RAW: trichomes before DGC, PURE: trichomes after DGC. Modified from Fig. 2 in [1].

Table 1
Data files shared in the PRIDE repository with accession PXD028808.

Data files shared in the ProteomeXchange repository with accession PXD028808			
Number	Sample	Replicate	File name
1	LEAF(+)	rep 3	210,312_A_MM_Aachen_1_TI0012_FM_03_GG4_1_1950.d.zip
2	LEAF(-)	rep 3	210,312_A_MM_Aachen_2_TI0012_DIS_03_GG5_1_1955.d.zip
3	RAW	rep 3	210,312_A_MM_Aachen_3_TI0012_RAW_03_GG6_1_1945.d.zip
4	PURE	rep 3	210,312_A_MM_Aachen_4_TI0012_PURE_03_GG7_1_1940.d.zip
5	LEAF(+)	rep 4	210,312_A_MM_Aachen_5_TI0012_FM_04_GG8_1_1951.d.zip
6	LEAF(-)	rep 4	210,312_A_MM_Aachen_6_TI0012_DIS_04_GG9_1_1956.d.zip
7	RAW	rep 4	210,312_A_MM_Aachen_7_TI0012_RAW_04_GG10_1_1946.d.zip
8	PURE	rep 4	210,312_A_MM_Aachen_8_TI0012_PURE_04_GG11_1_1941.d.zip
9	LEAF(+)	rep 1	210,312_A_MM_Aachen_9_TI0018_FM_01_GG12_1_1952.d.zip
10	LEAF(-)	rep 1	210,312_A_MM_Aachen_10_TI0018_DIS_01_GH1_1_1957.d.zip
11	RAW	rep 1	210,312_A_MM_Aachen_11_TI0018_RAW_01_GH2_1_1947.d.zip
12	PURE	rep 1	210,312_A_MM_Aachen_12_TI0018_PURE_01_GH3_1_1942.d.zip
13	LEAF(+)	rep 2	210,312_A_MM_Aachen_13_TI0018_FM_02_GH4_1_1953.d.zip
14	LEAF(-)	rep 2	210,312_A_MM_Aachen_14_TI0018_DIS_02_GH5_1_1958.d.zip
15	RAW	rep 2	210,312_A_MM_Aachen_15_TI0018_RAW_02_GH6_1_1948.d.zip
16	PURE	rep 2	210,312_A_MM_Aachen_16_TI0018_PURE_02_GH7_1_1943.d.zip

from all 16 samples (four materials x four replicates), digested with trypsin, purified and the resulting peptides analyzed by nanoLC-MS/MS using data-dependent fragment ion data acquisition on an Impact II QqToF-system (Bruker).

The tandem mass spectrometry raw data as well as exemplary analysis based on the database search results (MaxQuant output folder) have been made publicly accessible to the ProteomeXchange Consortium [6] via the PRIDE partner repository [7] with the dataset identifier PXD028808 (https://www.ebi.ac.uk/pride/archive/projects/PXD028808). This dataset includes 16 zip-compressed .d.zip files, each containing the mass spectrometry raw data of one of the 16 individual samples in the Bruker .d data format (Table 1). In addition, the dataset includes 1 zip-compressed folder with the MaxQuant search parameters as .xml file and the search results used for further analysis as .txt files.

The proteinGroups.txt file of the MaxQuant search lists 2756 identified proteins. In our analysis in the companion publication [1], we removed contaminant proteins and reverse hits, resulting in 2669 proteins, of which 2240 were quantified in at least three replicates of one of the experimental conditions. Application of stringent cutoffs (Students *t*-test q-value < 0.05, corrected for multiple hypothesis testing with a permutation-based false discovery rate (FDR) < 0.05, and at least two-fold change in abundance) identified 906 proteins with differential accumulation between PURE samples and LEAF(-) samples, of which 223 were significantly enriched in PURE trichomes (Supplementary Table 1).

3. Experimental Design, Materials and Methods

3.1. Experimental Design

We collected *A. thaliana* material at four distinct stages during the trichome enrichment procedure: (i) rosette leaves of 42 day old seedlings (LEAF(+)), (ii) remaining trichome-depleted rosette leaf material (LEAF(-)), (iii) corresponding trichomes separated by stirring in EGTA-PBS buffer (RAW) and (iv) purified trichomes after density gradient centrifugation (PURE). Two trichome isolation experiments each were performed from two independently grown batches of *A. thaliana* seedlings. All samples were stored frozen at -80 °C until further processed in parallel: Proteomes were extracted using denaturing SDS-buffer and prepared for nanoLC–MS/MS analy-

sis using a SP3 bead-based in solution digestion protocol with trypsin, with equal amounts of protein input material used for normalization.

3.2. Plant Material and Trichome Enrichment

A. thaliana wildtype plants (Col-0) were grown on soil through holes of a perforated metal plate to facilitate soil-free harvest. After 42 days of growth in controlled conditions (22/20 °C day/night temperature; 10 h light/14 h dark night cycle; photosynthetic photon flux density of 80–100 µmol m^{-2} s^{-1}; relative humidity of 80–90%), above-ground tissue was rapidly harvested with a razor blade, rinsed with tap water and dried with paper towel. Immediately after harvest, approximately 36 g of tissue were transferred to a 500 ml beaker containing 1 g of glass beads (diameter 250–500 µm) and 240 ml EGTA-PBS (50 mM EGTA solution (pH 8.0), 10 mM disodium hydrogen phosphate, 2.7 mM potassium chloride, 2.0 mM potassium dihydrogen phosphate, 0.5 mM magnesium chloride, 137 mM sodium chloride, pH 7.5). An aliquot of the *A. thaliana* source material was saved at this stage (LEAF(+)) before stirring with a magnetic stirrer (IKAMAG Combimag RCT, IKA) at 300 rpm for 30 min using an 80 mm triangular stirring bar. Four layers of screen door mesh were used as a first filter to separate the processed seedlings and the buffer solution containing the released trichomes. The stirred seedling material was rinsed with PBS to free trichomes trapped in the residual plant material, passed through the door mesh and combined with the first flow-through. The trichome suspension was then applied on a cell strainer with 100 µm pore size (VWR), restrained trichomes carefully collected with a small spatula and transferred to 2 ml reaction tubes. Enriched trichomes were stored at 4 °C, while the process of agitation and subsequent filtration was repeated twice, with 15 min agitation time and reusing the EGTA/EDTA-containing PBS buffer and plant material. After enrichment, trichome fresh mass was measured and trichomes were evenly distributed among two 2 ml reaction tubes. One aliquot was frozen as crude trichomes (RAW), along with an aliquot of the trichome-depleted seedlings (LEAF(-)). The second 1 ml aliquot of the trichome suspension was applied to a density gradient consisting of four layers with different sucrose concentrations (0, 20, 40, 80% (m/v); highest concentration at the bottom of the centrifuge tube) and centrifuged in a swing-out rotor at 3000 g for 10 min at room temperature. Subsequently, the layers were carefully removed and the bottom fraction transferred to 15 ml reaction tubes, diluted with PBS, mixed and centrifuged at 3000 *g* for 3 min at room temperature. The pure trichome pellet (PURE) was washed one more time with PBS and frozen until further protein extraction. For further details of the enrichment procedure see also [5].

3.3. Proteomics Sample Preparation

Frozen sample aliquots were subjected to lyophilization using an Alpha 1–2 LDplus freeze dryer (Martin Christ). Afterwards, 10–20 mg dry mass (corresponding to approximately 200 mg fresh mass) of each sample were ground to fine powder using a bead mill (Retsch), suspended in 100 µL protein extraction buffer (2.5 mM EDTA, 100 mM HEPES, 4% SDS) using a pipette and vacuum-infiltrated in a custom-made desiccator for 5 min. Cellular debris was removed by centrifugation at 10,000 *g* for 1 min at 21 °C. The proteome extract in the supernatant was transferred to a new 1.5-mL reaction tube and protein concentration assayed using the Pierce BCA protein assay kit (Thermo Fisher Scientific) with a calibration curve fitted to the average values of three replicates of a six-step bovine serum albumin (BSA) dilution series (0, 125, 250, 500, 1000, 2000 ng μL^{-1}) by second degree polynomial regression. Protein samples were then frozen and stored at -20 °C until further processing. Twenty microgram of the proteome was denatured by heating to 60 °C for 5 min and reduced by addition of 10 mM dithiothreitol (DTT) for 30 min at 37 °C. Cysteine residues were then alkylated by incubation with 50 mM chloroacetamide in the dark at 21 °C for 30 min. Next, 1 M DTT was added to a final concentration of

50 mM and the sample further incubated at 21 °C for 30 min to quench the reaction. For buffer exchange, single-pot solid phase (SP3) paramagnetic beads were used [8]. Proteins were bound to the beads by addition of nine sample volumes acetonitrile (ACN, 90% final concentration), washed with 90% ACN, and released by reconstitution in digestion buffer containing 100 mM HEPES and 2.5 mM $CaCl_2$ at pH 7.5. Trypsin was added in a protein:protease ratio of 100:1 and incubated for 18 h at 37 °C. The samples were acidified to pH<3.0 by addition of 1% formic acid and desalted by using self-packed double-layer C18-STAGE tips [9].

3.4. Mass Spectrometry Data Acquisition

An estimated 1 µg of desalted peptides were loaded on a Ultimate 3000 RSLCnano chromatography system (Thermo) operated in two-column setup with a µPAC reverse phase trap column (PharmaFluidics) and a 50 cm µPAC reverse phase analytical column (PharmaFluidics) operated at a column temperature of 40 °C. Peptides were eluted with a flow rate of 600 nl min^{-1} using a binary gradient from 2 to 30% eluent B (A: 0.1% formic acid in water, B: 0.1% formic acid in ACN) with 150 min effective separation time and a total runtime of 3 h per sample. Separated peptides were ionized using a CaptiveSpray nano-ESI source (Bruker) with the nitrogen gas saturated with ACN using a nanobooster (Bruker) and introduced to an Impact II high resolution QqTOF mass spectrometer (Bruker) as described [10]. Mass spectrometry data was acquired with the Bruker HyStar Software (v5.1, Bruker) in line-mode in a mass range from 200 to 1750 *m/z* at an acquisition rate of 5 Hz. The 14 most intense ions were selected for fragmentation, with fragment spectra automatically acquired between 5 and 20 Hz depending on the precursor intensity. Selected precursors were excluded for the next 0.4 min unless signal to noise ratio improved 3-fold.

3.5. Mass Spectrometry Data Analysis

For the analysis presented in our companion publication [1], peptides and proteins were identified by target-decoy spectrum-to-peptide matching with the MaxQuant software package [11] v. 2.0.1 in combination with the Uniprot [12] *A. thaliana* proteome database (release 2021_02) and a database of standard contaminant proteins provided with the MaxQuant release. Standard settings for Bruker QTOF instruments were used, with the exception of minimum MS1 intensity, which was set to 30. Trypsin was set as specific digestion enzyme, allowing for up to two missed cleavages. Carbamidomethylation of cysteine residues was considered as a fixed modification and protein N-terminal acetylation and methionine oxidation were considered as variable modifications. Due to the different proteome composition of the four samples, the match-between-runs-function was limited to matching between replicates of each four experimental conditions – (i) whole leaves (LEAF(+)), (ii) trichome-depleted leaves (LEAF(-)) and (iii) separated trichomes (RAW) as well as (iv) purified trichomes (PURE). MaxQuant LFQ values were calculated using standard settings, and a FDR of 0.01 was applied at the levels of peptide-sequence-matches (PSM) and protein identifications. We used the Perseus software package [13] v.1.6.15.0 for further processing. After loading the MaxQuant protein.txt and removal of proteins flagged as contaminants and reverse database hits, 2669 proteins were identified, of which 2240 were quantified in at least three replicates of one of the experimental conditions. For each experiment, missing values were imputed from a down-shifted normal distribution using standard Perseus settings. Comparison of proteins identified in PURE and LEAF(-) samples identified 906 proteins with significantly different accumulation (Student́s *t*-test *q*-value < 0.05, corrected for multiple hypothesis testing with a permutation-based FDR < 0.05, and at least two-fold change in abundance), of which 223 were significantly enriched in PURE trichomes (Supplementary Table S1).

Ethics Statements

The work was performed and data acquired under strict observation of the relevant ethics and work safety guidelines.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Arabidopsis thaliana trichome proteome (Original data) (PRIDE).

CRediT Author Statement

Jan W. Huebbers: Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing; **Melissa Mantz:** Investigation, Formal analysis, Writing – original draft; **Ralph Panstruga:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing; **Pitter F. Huesgen:** Data curation, Formal analysis, Resources, Supervision, Writing – review & editing.

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Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2023.108897.

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